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Parasitism, Family Conflict and Breeding Success

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Abstract

Parasites are important drivers of ecological and evolutionary processes in their hosts. However, hosts often differ in how they are affected by parasitism, which can be important in how parasite effects on individuals scale up to the population level. Hosts may differ intrinsically in their susceptibility to parasitism, and extrinsic factors may impose constraints on how hosts allocate resources between immunity, maintenance and reproduction, thereby further affecting their ability to cope with infection. These extrinsic factors include the host's ecological environment, for example food availability or weather, and its social environment, that is its interactions with conspecifics. This is particularly true during a reproductive attempt when individuals interact closely with other family members. Not only might immediate impacts of parasitism differ between and within parents and offspring, but the direct effects of parasitism on a host could have further indirect consequences for other family members through their behavioural interactions with parasitised individuals. The distribution of direct and indirect effects among all family members could affect the outcome of the breeding event and individuals' future performance. However, teasing apart these various avenues of parasite impacts on families may be difficult if parasite burden or susceptibility is correlated between family members. In this thesis, I explore the consequences of parasitism for different family members of the European shag *Phalacrocorax aristotelis* infected with gastrointestinal nematodes, over a range of ecological conditions.

In chapter 2, I demonstrate that chicks' responses to anti-parasite treatment across four years vary between siblings and with environmental conditions, which may be mediated by resource allocation among siblings. In chapter 3, I explore how costs of parasitism are distributed among the whole family by simultaneously treating chicks and/or parents with an anti-parasite drug and measuring the outcomes for all family members. Treatment has a more marked effect for the non-treated generation than for the treated individuals, suggesting that parasitism may have important indirect costs. In chapter 4, I investigate whether within-brood variability in the effects of anti-parasite treatment and its cross-generational impacts are mediated by behavioural change, and show that chick treatment but not parent treatment influences several aspects of behaviour in the nest. In chapter 5, I demonstrate that the impact of chick anti-parasite treatment on parents persists beyond the breeding attempt, with parents of treated chicks foraging less overwinter and breeding earlier the following year, whereas there is no persistent effect of parents' own anti-parasite treatment. Lastly, I provide an appendix examining the parasitology of the system in detail, including an assessment of *in situ* and proxy measures of worm burdens of chicks. This thesis demonstrates that parasitism can be a key component, previously overlooked, of reproductive performance in seabirds, a group that plays an important ecological role as apex predators and thus indicator species of the marine environment.

Lay Summary

Wild animals are almost always infected with large numbers of parasites. A parasite infection can be expensive for the host to deal with, leading to negative consequences such as less successful breeding or poorer survival. By altering these vital rates, effects of parasites on individual hosts have the potential to scale up and affect the dynamics of entire populations. However, different hosts suffer these effects to different extents. Intrinsically, some may be more able to cope with an infection, for example because they are in better condition or have more efficient immune systems. Hosts may also be subject to different extrinsic influences, including both their ecological environment, for example food availability, and their social environment, that is how they interact with other individuals. These differences between hosts could be especially important during breeding, when parents must weigh up how much to invest in their own parasite defences against investing in caring for their young, who may be particularly vulnerable to infection. In this thesis, I investigate how parasite infection of nestling chicks and their parents influences these trade-offs between family members and how this affects the outcome of the breeding attempt. I use experimental anti-parasite treatment of the European shag *Phalacrocorax aristotelis* to examine the role of gut parasites in chick growth and survival, behavioural interactions between chicks and their parents, parent condition and parents' behaviour during and after the breeding season. As a seabird, the shag is an important indicator of changes in the marine environment, and understanding its responses to these changes requires a full understanding of its ecology. Parasitism is an important part of this ecology that has until recently been overlooked.

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Declaration

I declare that I have composed this thesis, under guidance from my four supervisors. I conducted the majority of the fieldwork and other data collection, with help as detailed below, and all analyses are my own. This work has not been submitted for any other degree or professional qualification except as specified.

Chapter 2 includes data collected by Thomas Reed during his PhD (2006 and 2007 seasons), published as (Reed et al., 2012), as well as my own (2010 and 2011 seasons). My fieldwork in 2010 relied on help from Katherine Herborn and in 2011 from Emi Takahashi. The analysis of the combined four years is my own. Emi Takahashi also conducted the majority of the faecal egg counts presented in this chapter.

Chapters 3, 4 and 5 are based on the experiment I carried out in the 2011 season, with substantial fieldwork assistance from Emi Takahashi. Emi also carried out a large proportion of the molecular sexing of the chicks from this experiment.

All chick sexing (from experiments in **chapters 2 and 3**) was conducted using laboratory facilities provided by Josephine Pemberton.

Video analyses from 2010 in **Chapter 4** were conducted in part by Katherine Herborn.

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(*Hanna Granroth-Wilding*)

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CHAPTER 1

Introduction

It is believed that almost every wild animal is infected at some point in its life with parasites, which are often abundant and diverse (Clayton & Moore, 1997; Lafferty, 2010). Parasites derive their resources from another living organism, without providing any direct benefit to the host, but often have little obvious or visible impact on wild hosts. This fact, in addition to the ubiquity of parasites in ecosystems, led early pioneers in the field of evolutionary ecology to overlook parasitism as a factor that might shape an animal's ecology and life-history decisions (Gulland, 1995). Indeed, David Lack considered parasite infection to be inconsequential to the host (Lack, 1968). Instead, emphasis was placed on processes with more obvious costs or benefits such as predation, foraging and reproduction. In the last four decades, however, it has become clear that parasitism is a crucial aspect of evolutionary ecology. Initially, research focused predominantly on lethal or severe, acute impacts, typically associated with pathogenic microparasites (e.g. Anderson & May, 1978; Hamilton & Zuk, 1982), and attention tended to fall on epidemic outbreaks of disease. The ecological importance of parasitism was considered predominantly at the population level, examining the consequences of altered demographic rates in relation to parasite fitness traits such as transmission and virulence (Anderson & May, 1978; May & Anderson, 1979; Hudson et al., 1992), rather than on how it might influence individual life-history decisions in hosts. Only in the last two decades has an appreciation arisen of the importance of naturally

occurring endemic infections for individual fitness in wild hosts, not only in terms of survival but also through sub-lethal effects on fitness components such as reproductive success (Grenfell & Dobson, 1995; Gulland, 1995; Hudson et al., 1998; Telfer et al., 2002; Burthe et al., 2008) which are typical of macroparasite infections (Clayton & Moore, 1997; Hudson et al., 2002). However, much remains to be learnt about how the demands of a parasite infection might interact with other drivers of individual ecological and life-history processes, such as those traditional considerations of foraging and reproduction as well as external environmental influences. This is particularly the case for endoparasites, due in large part to the difficulty of quantifying endoparastic infection intensity. Because this usually requires destructive sampling of hosts, much of our understanding of the role of parasites such as nematodes for ecological processes in their host is based on cross-sectional data (e.g. Hudson et al., 1992; Lello et al., 2004; Newey et al., 2005), limiting our appreciation of how endoparasitism may affect host life-history. Treatment studies offer an alternative approach, allowing parasite burdens to be manipulated and the consequences monitored for individual hosts (e.g. Hudson et al., 1998; Pedersen & Greives, 2008).

The negative effects of parasitism do not fall equally on all hosts. Individuals may vary in their exposure to infection, their immune defences to prevent parasites from establishing or proliferating, or their ability to deal with the costs of a given infection (Shaw & Dobson, 1995; Norris & Evans, 2000; Zuk & Stoehr, 2002). This variability at all stages of an infection can be attributed to effects both intrinsic to the host, such as its immunocompetence or overall phenotypic quality, and extrinsic, such as food availability or its interactions with other individuals (Schmid-Hempel, 2003; Sandland & Minchella, 2003). Impacts of parasites on individual hosts may, therefore, have different implications for population processes depending on which individuals are affected, the composition of the population and the prevailing environmental conditions. In this thesis, I investigate how such variability influences the impact of parasites during a reproductive attempt, a crucial time for life-history and physiological trade-offs, when individuals may also be influenced by the behaviour of other family members (fig. 1.1).

This introductory chapter begins with an overview of the ways in which individuals may be impacted by a parasite infection, how these impacts might vary between individuals, and why hosts might be particularly vulnerable during early development (section 1.1). I then describe another substantial factor in early development, conflicts of interests between family members, and how these influence investment decisions during a reproductive attempt (section 1.2). I then bring these two aspects together to

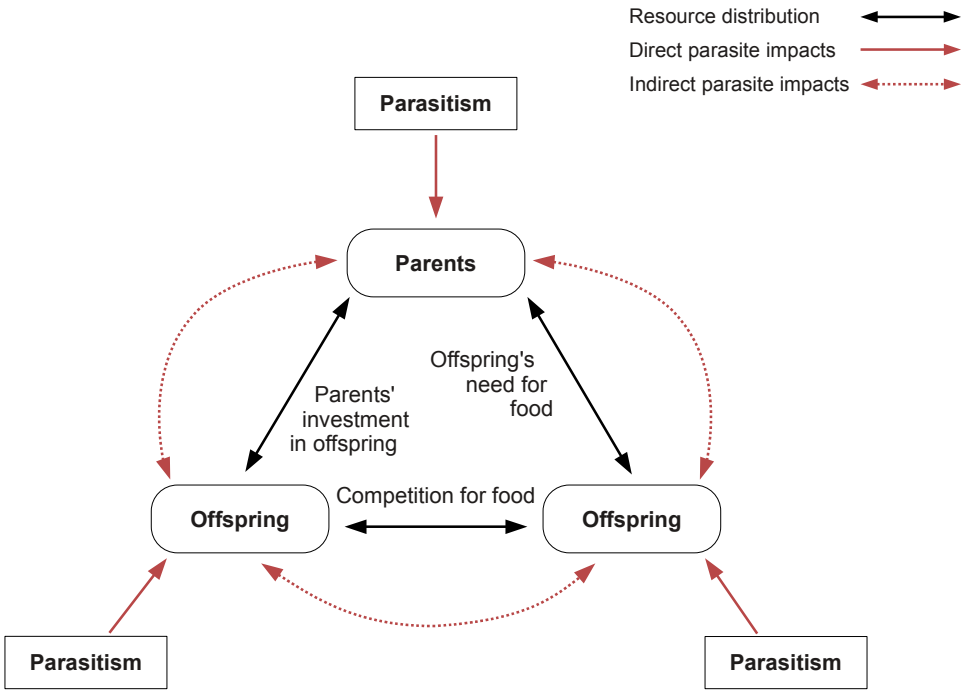


Figure 1.1: In addition to having variable direct effects on infected hosts, parasitism might interact with resource allocation patterns within the family to indirectly affect other family members.

describe the importance of considering parasitism during reproduction within a context of family conflict, outlining the findings and limitations of current research in this field (section 1.3). Lastly, I introduce my study system, the European shag *Phalacrocorax aristotelis* and its gastrointestinal nematode parasites (section 1.4) and outline the aims of this thesis (section 1.5).

1.1 Costs and consequences of parasitism for hosts

Obvious signs of sickness or pathogenesis are typically associated with certain microparasite infections that multiply directly inside a host, such as viruses, bacteria and protozoan blood parasites (Clayton & Moore, 1997; Hudson et al., 2002). The general pattern for macroparasites such as nematodes and biting arthropods, on the other hand, is for chronic infections with few clinical signs (Clayton & Moore, 1997; Hudson et al., 2002). Indeed, in their seminal paper on the impact of parasitism on host population dynamics, Anderson & May (1978) state that macroparasites “appear to do

very little harm to their hosts", later modelling the inflluence of macroparasites on host population dynamics via their impact on host birth and death rates (May & Anderson, 1979). Similarly, in an early study demonstrating a cost of biting ectoparasites on a wild population (cliff swallows *Hirundo pyrrhonota*), Brown & Brown (1986) suggest that systems in which parasites have persistent deleterious effects on their hosts may be rare. In contrast, the costs of parasitism in domestic livestock were well accepted as early as the 1960s because of their detrimental effect on productivity of milk, meat or other commodities (reviewed in Sykes, 1994). However, from the 1990s, an ever-increasing body of empirical studies has brought about an acceptance that macroparasites are costly to wild hosts and thus potentially powerful drivers of ecological and evolutionary processes (Loye & Zuk, 1991; Clayton & Moore, 1997; Hudson et al., 2002; Tompkins et al., 2011). Even non-lethal impacts can alter hosts' resource use, potentially affecting many aspects of host performance and hence ecology, behaviour and life-history. In this thesis, I focus on macroparasites, and the term "parasite" generally refers to macroparasites unless specified.

The costs that both macro- and micro-parasites impose on their hosts can be summarized as due to three main factors. Firstly, hosts invest in immune responses against parasites to prevent initial infection and clear or control an established infection. Immune responses are costly because of the energy and nutrient requirements in producing and/or activating immune cells and immunoglobulins, as has been widely demonstrated (reviewed by Hasselquist & Nilsson, 2012), and thus trade off against other physiological processes such as growth or reproduction (e.g. Soler et al., 2003; Råberg et al., 2000, respectively). These trade-offs, along with variation between individuals in their ability to mount an immune response, make immunity a crucial aspect of resource allocation within an individual and thus an important eco-evolutionary consideration (Sheldon & Verhulst, 1996; Zuk & Stoehr, 2002; Norris & Evans, 2000; Hasselquist, 2007; Demas & Nelson, 2011). Secondly, there may be costs in terms of tissue damage and subsequent repair from pathology caused by the parasite. This can increase the host's metabolic rate and hence its resource requirements (Colditz, 2008). Thirdly, macroparasites in particular may directly deprive the host of resources (Atkinson et al., 2009), for example gastrointestinal helminths taking up nutrients that the host has ingested. The relatively large size of macroparasites means that they may make up considerable biomass and could consequently have high resource requirements relative to smaller but more pathogenic parasites. In concert with immune investment and tissue repair, this withdrawal of resources raises the risk of anorexia in

the host, particularly in the case of gastrointestinal nematode infection (Colditz, 2008). In addition, macroparasites may take a long time to reach maturity and are thus under selective pressure to avoid host detection and to keep the host alive and functioning (Hudson et al., 2002). Macroparasites therefore have the potential to influence hosts' behaviour and life history decisions for extended periods of time.

Altogether, therefore, parasitic infection is expected to influence the host's priorities for allocation of nutrients, redirecting resources from other physiological processes, maintenance functions and reproductive investment. In wild animals, these direct costs can have myriad ecological consequences. Gastrointestinal nematode abundance in red deer *Cervus elaphus*, for example, is negatively associated with condition (mass and visceral fat) in culled adults (Irvine et al., 2006), and in sage thrashers *Oreoscoptes montanus*, infection of chicks with ectoparasitic blowfly larvae increases chick mortality in inclement weather (Howe, 1992). Although these correlational findings could also be interpreted as poorer-quality hosts being more vulnerable to infection, giving rise to an apparent causal relationship of parasitism decreasing host fitness, parasite removal experiments overwhelmingly support an ecological cost of parasitism for wild hosts (summary table in Tompkins & Begon, 1999). Macroparasitism has been shown to reduce condition, fecundity, survival and future reproduction in many wild populations, including reindeer, great tits, European shags and Alpine swifts (Stien et al., 2002; Richner et al., 1993; Knowles et al., 2010a; Reed et al., 2008; Bize et al., 2004). By altering crucial demographic traits, parasite infection has the potential to lead to population-level effects. Indeed, parasite removal experiments have indicated a role for infection in regulating wild populations of red grouse *Lagopus lagopus scoticus* (Hudson et al., 1992), mountain hares *Lepus timidus* (Newey et al., 2005) and reindeer *Rangifer tarandus* (Albon et al., 2002). However, it has proved difficult to ascertain whether parasites *per se* exert a strong enough ecological force to lead to population changes, or whether they act in concert with other extrinsic influences to exacerbate population changes driven by other factors (Newey et al., 2005; Redpath et al., 2006; Tompkins et al., 2011).

1.1.1 Variability in impacts of parasites

Individual hosts may vary considerably in the burdens of parasites that they carry and in how they are affected by those burdens (Schmid-Hempel & Koella, 1994; Shaw & Dobson, 1995). In addition to random genetic variation in immunocompetence, certain

traits may predispose a host to suffer negative impacts of parasitism. It has been suggested, for example, that testosterone can be immunosuppressive in some conditions, such that males may be more suscpetible to infection and its detrimental effects than females (Folstad & Karter, 1992; Roberts et al., 2004; Redpath et al., 2006). Parasite infection is also likely to be more detrimental to hosts that are already in poor physical condition, having fewer resources to direct to anti-parasite defence and suffering a greater marginal cost of the direct loss of resources to the parasite (Coop & Kyriazakis, 2001; Hoberg, 2005). In addition, in wild populations, environmental conditions are likely to shape the impact of a parasitic infection by affecting the condition of the host. In Eurasian kestrels *Falco tinnunculus*, experimental reduction of parental effort decreased malaria infection intensity only in years of low food availability, and environmental conditions have been suggested to act additively with nematode infection at the population level (Newey et al., 2005; Redpath et al., 2006). However, very few studies incorporate quantitative measures of environmental variability when assessing the impact of parasites at the level of individual hosts (Sandland & Minchella, 2003; Wolinska & King, 2009; Boughton et al., 2011), even though such interannual differences have been observed in several experimental studies on wild populations (e.g. Heeb et al., 1999; Stien et al., 2002; Knowles et al., 2010b).

Parasite impacts on individual hosts are most often considered in terms of their immediate physiological costs. However, parasitism can also affect host behaviour. There are many charismatic descriptions of parasites directly changing host behaviour in order to increase the likelihood of transmission (Poulin, 2010), and several authors have noted the presence of behavioural strategies in hosts to avoid infection with parasites (Hart, 1997) or to self-medicate if infected (e.g. Kacsoh et al., 2013; Suarez-Rodriguez et al., 2013). However, through its effects on resource allocation, parasitism could also influence host behaviour that is part of its normal repertoire. Altering behaviours such as foraging or provisioning young could have immediate and longer-term fitness implications for the host, and also raise the potential for individuals to be affected by parasitism in others via behavioural interactions.

1.1.2 Parasitism and early development

Conditions during early development can have profound consequences for any organism (reviewed by Lindström, 1999; Metcalfe & Monaghan, 2001; Monaghan, 2008), and parasitism is an important component of the developmental environment. Un-

favourable conditions in early life are believed to mechanistically impair growth and development, resulting in potentially life-long reductions in fitness (Monaghan, 2008). Perturbation to growth trajectories during early development has been shown to affect locomotor performance, attractiveness to mates, problem-solving ability and many other performance traits (Metcalfe & Monaghan, 2001; Monaghan, 2008). In particular, compensatory fast growth after a period of slow growth in poorer conditions ("catch-up growth") may have detrimental long-term effects, for example reducing reproductive performance and lifespan in sticklebacks *Gasterosteus aculeatus* (Lee et al., 2012, 2013). If the cost of dealing with a parasite infection diverts resources from growth and maintenance during early development, it could conceivably have life-long consequences for the host.

Young animals may also be more susceptible to the immediate effects of parasitism and the costs it imposes (Hudson & Dobson, 1997; Møller, 1997; Sol et al., 2003). The immune systems of young vertebrates are often not fully developed at birth or hatching, such that young animals may be less able to keep an infection under control (Wakelin & Apanius, 1997; Frank, 2002). Furthermore, in many species, early development is a vulnerable time, as evidenced by the general pattern of higher mortality rate of juveniles than of adults across a broad range of species (Stearns, 1992). Among vertebrates, juveniles may be more vulnerable to external stressors, for example to temperature fluctuations because of their small size or to predation because of their underdeveloped motor skills. Moreover, young animals invest heavily in growth, so may be less buffered against resource limitation than adults. Thus, a parasite infection that adds yet more costs at this vulnerable time might be more likely to have detrimental consequences for juveniles. In combination with the potential for conditions in early life to have long-lasting effects, differences between juveniles in how they cope with infection could lead to differences in performance later in life. Many studies have illustrated negative immediate impacts of parasitism for juveniles, with a focus on bird/ectoparasite systems, in terms of reduced chick survival, slower growth or delayed fledging success (e.g. Brown & Brown, 1986; Richner et al., 1993; Bize et al., 2004). Longer-term effects of parasitism in early life have also been demonstrated: in great tits *Parus major*, for example, flea infection during development can reduce males' song duration once mature (Bischoff et al., 2009), and reduce the dispersal distance and future reproductive output of both sexes (Heeb et al., 1999; Fitze et al., 2004a).

How a young animal deals with a parasite infection is also likely to depend on its intrinsic susceptibility to infection and ability to deal with its negative effects, as well as on other extrinsic aspects of its developmental environment that bring resources or costs. An important aspect of this susceptibility is the cross-generational transfer of immunity: mothers can transfer antibodies to the offspring via the egg, in birds, or in mammals via the placenta or milk, and there is evidence for cross-generational immune transfer in invertebrates, though the mechanisms are less clear (Mousseau & Fox, 1998; Glezen, 2003; Mousseau et al., 2009; Little & Kraaijeveld, 2004). In birds, maternal antibodies have been shown to be functional against macroparasites (Buechler et al., 2002; Gallizzi et al., 2008b; Gallizzi & Richner, 2008; but see Tschirren et al., 2005) and to persist for a considerable part of chick-rearing across species (Garnier et al., 2011). Parasite infection of mothers can trigger antibody production and thus antibody transfer to eggs, improving offspring defences against parasites they are likely to encounter early on, although it has been suggested that maternal antibodies may also interfere with the development of offspring's own immunity (reviewed in Boulinier & Staszewski, 2008). In birds, the pattern of maternal allocation of antibodies commonly varies through a clutch, such that siblings might differ in their ability to defend themselves against infection and its detrimental effects. However, no general pattern in the direction of this variation has yet been established: for example, in collared flycatchers *Ficedula albicollis*, eggs laid later in the clutch contain higher concentrations of antibodies (Hargitai et al., 2006), whereas in the black-headed gull *Larus ridibundus*, antibody allocation decreases through the clutch (Groothuis et al., 2006). Mothers may also invest other substances in the embryonic environment, such as hormones, which could indirectly affect offspring's ability to cope with an infection, for example by altering offspring's competitive ability (Groothuis et al., 2005; Müller et al., 2007; Dantzer et al., 2013). These may also vary through the clutch, again with no obvious general rule: in a single study, Schwabl (1993) found increasing yolk testosterone concentrations, associated with competitive ability, through clutches in canaries *Serinus canaria*, but no difference with laying order in zebra finches *Taeniopygia guttata*. Intrinsic asymmetries between siblings may also arise at birth if offspring are born or hatch asynchronously, common among birds, leading to age and thus size differences in the brood (Stenning, 1996). Younger brood members are often competitively inferior to their older siblings, and may thus have less access to food or other aspects of parental care (Drummond, 2006).

An offspring's development will also depend on extrinsic influences beyond these within-brood intrinsic asymmetries. Extrinsic factors can be considered in two broad groups: the broader ecological and abiotic environment, governing factors such as weather conditions and food availability, and the social environment of the nest, i.e. a chick's interactions with its siblings and parents. Poorer environmental conditions are likely to impair development but may also interact with intrinsic differences between chicks such that not all siblings suffer to the same extent. For example, younger, competitively disadvantaged chicks generally have higher mortality in poor conditions, making more food available to the older siblings who may thus be relatively unaffected by the environmental conditions (Mock & Forbes, 1995; Bonabeau et al., 1998; Forbes, 2009). In addition, for altricial species, offspring's early development is heavily reliant on parental care in forms such as food, protection and thermoregulation (Royle et al., 2012). Parents may be selective about which offspring they care for, and siblings may compete with each other for care (Mock & Parker, 1997). The importance of the social environment to chick development is illustrated by Forbes (2011) in red-winged blackbirds *Agelaius phoeniceus*, where the outcome of an initial handicap for chicks (in terms of hatching order) is determined by the social structure of the brood. In a family of four, a fourth-hatched chick had 80% chance of survival if it hatched within a day of its siblings, but only 38% if it hatched a day later and thus more subordinate. Similarly, the extent to which an individual brood suffers negative impacts of parasitism may be strongly shaped by the way in which it interacts with its family members (fig. 1.1).

1.2 Intra-familial conflict

Intra-familial behavioural interactions are a core question in behavioural and evolutionary ecology. Patterns of parental provisioning of offspring and competition between siblings have attracted the attention of naturalists since Aristotle, who notes in his *History of Animals* that in swallows, "both the male and female labour in support of the young. They feed each in turn, observing by some agreement the one which was first fed, that none may receive food twice". The idea of an agreement between parents and offspring remained the dominant paradigm for over 2000 years (e.g. Lack, 1947). Trivers (1974) overturned this to build the framework of our current understanding of conflict between family members being the driving force in the evolution of family life. By combining Hamilton (1964a,b)'s genetic concepts of inclusive fitness and kin selection with the ecological cost and genetic benefit of reproduction, Trivers (1974)

showed that offspring should, from an evolutionary perspective, demand more care for themselves than is optimal for parents to give or siblings to allow. In most vertebrates (sexual, diploid organisms), siblings share approximately half of their genetic material with each other and with each of their parents. Thus, an offspring gains twice as much fitness from a unit of parental investment in itself than its parents or its siblings do. Hence, each family member's optimal pattern of resource allocation among the family is, in evolutionary terms, skewed towards itself.

Family conflict may not be confined to a given reproductive attempt (intra-brood conflict). Williams (1966) was the first to suggest that reproduction in iteroparous species comes at a cost to a parent's residual reproductive value. Current reproductive investment therefore trades off against investment in future reproductive attempts (Trivers, 1972). Investment in a current brood may thus be more profitable to the chicks in that brood than to the parent, balancing this against reproductive success it might achieve in the future. Hence, siblings in a current brood are not only in conflict with each other and their parents, but also with past and future siblings (interbrood conflict). Family conflict in all its forms has been studied in a range of taxa, including mammals and insects (reviews in Mock & Parker, 1997; Hudson & Trillmich, 2007; Kilner & Hinde, 2008), but in wild populations, the majority of attention has focused on birds, and in this overview I will use mainly bird studies.

1.2.1 Conflict-related behaviour

Already at egg production, there are conflicts between parents' and offspring's interests. In many species, clutch sizes are larger than the number of chicks eventually fledged (Lack, 1947). This phenomenon is termed overproduction, which is then followed by chick death, or brood reduction, either accidental or adaptive for the remaining chicks and/or for parents (reviewed in Mock & Forbes, 1995). Hatching asynchrony is believed to be closely tied to overproduction as it facilitates brood reduction by generating differences in offspring value to parents across the brood (Stenning, 1996; Forbes, 2009). More specifically, hypotheses for the evolution of hatching asynchrony fall broadly into two categories (Mock & Forbes, 1995). The later-hatched ("marginal") offpsring may function as insurance against the accidental failure of older, more valuable ("core") offspring (Forbes & Lamey, 1996). In species with obligate siblicide, the insurance offspring can only survive if the core offspring do not, as the older chicks kill their younger siblings (Mock & Parker, 1997). Alternatively,

marginal chicks may serve a resource-tracking function, being forfeited first by parents in poor conditions to minimize wasted investment but surviving when conditions are favourable (Temme & Charnov, 1987). These hypotheses are not mutually exclusive and may apply to different extents in different species (Mock & Forbes, 1995).

In species with parental care, intra-familial conflicts during chick-rearing often play out in behavioural interactions in the nest. In particular, complex behaviours governing the distribution of resources within the family are understood to have evolved as a consequence of the genetic conflict between family members (Godfray, 1995; Kilner & Hinde, 2008; Royle et al., 2012), although behavioural conflict may not necessarily be evidence of evolutionary conflict (Mock & Parker, 1997). In this thesis, I focus on conflict between parents and offspring and conflict between siblings, acknowledging that sexual conflict between parents over the provision of care is another important and well-documented component of intra-familial dynamics (Royle et al., 2012). Phenomena such as the patterns of compensation when one parent's care effort is reduced, or mothers and fathers favouring different offspring, have been received much theoretical and empirical attention (McNamara et al., 1999; Lessells, 2002; Kilner, 2002; Harrison et al., 2009), but because the intra-familial dynamics of European shags have not been characterised in detail, these intricacies are beyond the scope of this thesis. Previous work in this system has, however, shown differences between siblings in response to external conditions (Stokland & Amundsen, 1988; Daunt et al., 1999; Reed et al., 2012), indicating that competition between siblings for parental provisioning is an important feature of breeding success in shags, so I address primarily parent-offspring conflict and sibling conflict in this study. Both have the potential to directly shape behaviour both between chicks and parents and among siblings (Godfray, 1995; Mock & Parker, 1997; Kilner & Hinde, 2012). In many birds, the most striking aspect of intra-familial conflict behaviour is the often extravagant signals of chicks to parents, using postural, vocal and other displays to indicate their desire to be fed (Wright & Leonard, 2002). This begging is proposed to be energetically costly for individual chicks and, therefore, to provide parents with useful information on how to distribute resources among the brood most profitably (Mock & Parker, 1997; Wright & Leonard, 2002).

Two contrasting hypotheses have been proposed to explain the evolution of these signals and parents' responses to them, which ultimately decide how resources are allocated among a brood. The first holds that begging displays are honest signals of need, with chicks in poorer condition begging more intensely to obtain more food, where the cost of the signal maintains its honesty according to the handicap principle (Za-

havi, 1975; Grafen, 1990; Godfray, 1991). This broadly accepted paradigm is founded on theoretical models explored by Godfray (1995), Kilner & Johnstone (1997) and Godfray & Johnstone (2000), which demonstrate such signalling to be an evolutionarily stable outcome of parent–offspring conflict (Kilner & Hinde, 2008; Grodzinski & Johnstone, 2012). The second hypothesis proposes that signalling is driven by competition between siblings rather than by genetic conflict between parents and offspring (Mock & Parker, 1997; Parker et al., 2002; Royle et al., 2002; Mock et al., 2011). Under this paradigm, begging is a signal of quality (as in sexual signalling theory, Zahavi, 1975), with chicks in better condition begging more intensely, and parents gain from feeding chicks that are most likely to survive (Mock & Parker, 1997; Mock et al., 2011). Proponents of this hypothesis criticize the first hypothesis (signal of need) for not giving sufficient consideration to other social interactions within the family, all of which should act simultaneously to affect the evolution of all intra-familial behaviour (contrast Godfray & Parker (1992) with Parker et al. (2002)). Certainly, competition between siblings in forms other than the begging display appears to be very important in many species in determining siblings' access to food, for example by larger, dominant siblings physically preventing younger siblings from feeding (Mock & Parker, 1997; Drummond, 2006). It is difficult to imagine that the evolution of such behaviour would be detached from the evolution of chick signalling and parental provisioning decisions (Mock & Parker, 1997). Indeed, Bonabeau et al. (1998) demonstrate theoretically that sibling competition alone can lead to a partitioning of parental provisioning that maximizes parents' fitness in the outcome of the breeding attempt. However, the evolutionary stability of signal honesty under the signal of quality hypothesis has been difficult to ascertain theoretically (Kilner & Hinde, 2008). In addition to begging, siblings often interact with each other through direct physical competition, which may also contribute to resource distribution patterns (Drummond, 2006; Hudson & Trillmich, 2007). The extent of such competition varies from jostling for position in the nest to be close to parents' feeding positions (e.g. Kölliker & Richner, 2004) to outright aggression, which in some species serves to establish and maintain behavioural hierarchies governing access to food (Rodriguez-Girones et al., 1996; Drummond et al., 2003) and in others leads to the death of subordinate chicks (Mock & Parker, 1998).

Despite decades of theoretical and empirical work in this field, experimental data on the role of begging rarely conform precisely to a single theoretical approach (Mock et al., 2011; Kilner & Hinde, 2012). Both hypotheses can predict similar observable

outcomes (Royle et al., 2002), and offspring signalling and sibling competition may interact very closely under either hypothesis (Smiseth et al., 2007). In addition, a growing body of evidence suggests external factors and other life-history trade-offs may affect the outcome of conflict-related behaviour in the nest. For example, parents of the hihi *Notiomystis cincta* with greater future reproductive potential are less responsive to chick signals (Thorogood et al., 2011), and in blue-footed boobies *Sula nebouxii*, when chicks are in sufficiently poor condition begging ceases to increase with need (Villaseñor & Drummond, 2007). Parents may even change their provisioning rules with environmental conditions: in both European starlings *Sturnus vulgaris* and Alpine swifts *Apus melba*, parents breeding earlier in the season preferentially provisioned chicks in worse condition, as indicated by the UV reflectance of their plumage, whereas late nesters favoured chicks in good condition (Bize et al., 2006). Parental decision-making in provisioning chicks and the resolution of intra-familial conflict thus remain open questions in evolutionary ecology.

1.3 Parasites and intra-familial conflict

I have described how parasites are costly in terms of resources, particularly in early life, and that a young animal's developmental environment depends on the decisions of family members in conflict over resource distribution. Parasite infection of all family members may, therefore, have a substantial role to play in the outcome of a breeding event and in parents' life-history decisions. However, the interplay between parasitism and family conflict has been investigated only by a limited number of researchers, and there is as yet no cohesive framework integrating these two crucial aspects of host ecology.

Several studies have illustrated empirically that parasitism and family conflict may indeed interact by demonstrating that parasitism in certain family members can affect others. Parasitised chicks may require increased parental effort, increasing parents' investment in a current reproductive attempt. In great tits, removing ectoparasites from chicks increased their begging and fathers increased provisioning rates in response (Christe et al., 1996). A similar study in blue tits *Cyanistes caeruleus* showed, however, that there was no apparent cost to parents in terms of physical condition of increased feeding of parasitized chicks (Tripet & Richner, 1997). In Alpine swifts, ectoparasitism of chicks delayed fledging, at a cost to parents' future reproduction (Bize et al., 2004). Parasitism in parents may also affect offspring, irrespective of their chicks'

infection status. The cost of their own parasite burden may require parents to divert resources away from reproduction to mounting an immune response or maintaining condition in the face of resource-demanding parasites. Adult house martins *Delichon urbica* treated for malaria infection increased their reproductive output (Marzal et al., 2005), and a similar study on blue tits suggests that such effects could be mediated by parasitized parents investing less in provisioning their chicks (Knowles et al., 2010a). In addition, theoretical models have shown that parasite infection of parents has the potential to influence the trade-off between current and future reproduction, if infection increases the cost of a reproductive attempt and parasitized parents thus strategically alter their investment in current offspring (Forbes, 1993). These studies all suggest that family conflict may be a key element in how individuals are impacted by parasitism, yet most studies investigating the impact of parasites during reproduction in wild populations only examine effects for certain family members or only superficially account for interactions with others. Furthermore, parents' parasite infections may have direct consequences for infection in the offspring via cross-generational transfer of immunity, as previously discussed.

Parasitism has also been suggested to interact with sibling competition. A selection of studies have documented differences between siblings in their response to parasite removal due to hatching order, size or sex (Reed et al., 2008; O'Brien & Dawson, 2009; Knowles et al., 2010a; Reed et al., 2012). This could be due to differences between siblings in their vulnerability to the negative effects of parasitism, due for example to patterns in maternal antibody investment or size asymmetries. Some chicks may in addition be more desirable to parasites, attracting infection and thus lessening the impact on the rest of the brood, as has been suggested for ectoparasites in passerine broods (Christe et al., 1998; Roulin et al., 2003; O'Brien & Dawson, 2009). In addition, the consequences of parasitism for chicks may also vary with the chicks' value to their parents. For example, if older siblings are more likely to survive and thus more profitable for parents to invest in, later-hatched chicks may suffer greater costs of infection if they do not receive sufficient resources to counter the infection. This could explain the findings of Reed et al. (2012) that anti-parasite treatment increased the growth rate of the youngest chick in broods of European shags *Phalacrocorax aristotelis*, but not its older siblings. However, while asymmetries between siblings in competitive ability or value to parents are a core aspect of the family conflict literature, parasite manipulation experiments rarely take them into account. Instead, intra-familial conflict behaviour is often offered as a post-hoc explanation for unexpected or complex physiological con-

sequences of parasite manipulation, without a direct examination of behaviour (e.g. Richner et al., 1993; Szép & Møller, 1999; Knowles et al., 2010a; Reed et al., 2012).

1.3.1 Limitations of research to date

Despite the body of literature investigating the role of parasites in host life-history decisions and family life, there is as yet no overall understanding of how parasitism interacts with other costs, constraints and decisions during a host's reproductive event. This is due in part to the complexity of the question, but also in part to heterogenous approaches, questions, methods and study systems in this field.

A substantial difficulty in synthesizing current knowledge is that many studies to date only consider the direct effects of treatment on the individuals whose parasite burdens are manipulated. Although cross-generational impacts have been demonstrated in many systems, the family-wide effects of parasites have yet to be fully explored. Moreover, parasite burdens are likely to be correlated between family members, especially in the commonly used systems of nest-based ectoparasites of passerine birds where parasites can move freely between family members in their shared nest environment. This horizontal transmission may mean that experimental manipulations of the parasite burden of certain family members also causes changes in the burdens of individuals whose parasites have not been directly manipulated. Despite demonstrations that parasite burdens can be correalted between treated and non-treated family members (Møller, 1994; Bize et al., 2004; Fitze et al., 2004b), they are rarely accounted for in experimental designs or interpretations of the results (but see Gallizzi et al., 2008a). In addition, few parasite manipulation studies examine the long-term effects for either parents or chicks of parasitism in the nest. Fitze et al. (2004a,b) provide a rare exception, showing that ectoparasitism of chicks reduces their own lifetime reproductive output and their parents' dispersal distance for the subsequent breeding season (see also Heeb et al., 1999; Bize et al., 2004). However, it remains unclear whether these effects of manipulation of chick parasitism could also be due to a correlated change in parents' parasite burden and direct impacts of that change.

Furthermore, the current body of knowledge relies heavily on a restricted set of species, using predominantly passerines and their biting ectoparasites, which raises four main concerns about the interpretation and collation of experimental results to date. Firstly, as these birds are relatively short-lived, they may tend to invest highly in each reproductive attempt and thus respond to external influences, such as parasitism,

in a very different way to longer-lived species that have more to gain by preserving their residual reproductive value (Stearns, 1992). Secondly, they tend to have large broods, making it very difficult to fully consider interactions between all family members. Incorporating a general framework of intra-familial interactions into our understanding of parasite impacts may, therefore, not be tractable in these species. Thirdly, the horizontal transmission of ectoparasites makes it difficult to untangle the relative importance of chick and parent parasite infection for the performance of all family members. Finally, the majority of experimental systems use ectoparasites (fleas, lice and ticks) or microparasites (malaria), leaving endoparasites underrepresented. These can make up a substantial part of a bird's parasite fauna (Atkinson et al., 2009), with infections of hundreds of individual worms not uncommon in many species (Hoberg, 2005; Atkinson et al., 2009). In combination with their direct removal of resources from the host's digestive tract, the cost of infection may be considerable. Moreover, endoparasites may be more difficult to avoid than ectoparasites, particularly if transferred through food, and difficult to remove, as immune responses are the only defence once the parasite is established, unlike behavioural strategies such as preening against ectoparasites (Hart, 1997).

1.4 Study system

In this thesis, I investigate the interaction between parasitism and family conflict in the European shag, using anti-nematode treatment to examine how the impacts of infection on individuals are influenced by intrinsic and extrinsic variability. The shag is a piscivorous seabird, around 2kg in weight, endemic to the European Atlantic coast and the Mediterranean (Wanless & Harris, 1997). They are prominently infected with gastrointestinal nematodes, with 100% of adult shags hosting worms and considerable variation in burdens between individual hosts (Abollo et al., 2001; Burthe et al., 2013). Nematode infection of seabirds in general has been suggested to be important in exacerbating the negative effects of unfavourable environmental conditions (Hoberg, 2005), but has received little attention beyond occasional studies and anecdotal observations (discussed in appendix A).

Shags are unusual among seabirds in displaying relatively large variation between years in fecundity and survival, or "boom–bust dynamics", throughout their breeding career (Frederiksen et al., 2008). This reflects variation in various environmental factors including food availability, sea temperature and climate (Aebischer & Wanless,

1992; Frederiksen et al., 2007b; Burthe et al., 2012), and both survival and fecundity are sensitive to winter weather as shags carry only small fat reserves (Aebischer & Wanless, 1992; Daunt et al., 2006a; Frederiksen et al., 2008). As well as being susceptible to population crashes, shags have the potential for rapid population growth (Frederiksen et al., 2008) as they are able to raise up to three (exceptionally four) chicks each year (Harris et al., 1994). Factors affecting these key demographic variables thus have the potential to rapidly be reflected at the population level, and gastrointestinal parasitism with its associated risk of anorexia (Colditz, 2008) could be crucial. As yet, however, the role of parasitism in population processes has been relatively little investigated in shags or seabirds at large (Skorping, 1996; Galaktionov, 1996; Hoberg, 2005).

Three species of nematode have been identified in shags to date: *Contracaecum rudolphii*, *C. septentrionale* and *Anisakis simplex* (Abollo et al., 2001; Reed et al., 2008; Burthe et al., 2013). These are all anisakid ascaridoid worms that pass through two paratenic hosts (small crustaceans and fish) before infecting the shag as third-stage larvae through the fish that it eats (Anderson, 2000; McClelland, 2005; Fagerholm & Overstreet, 2008). There are several suggestions that the nematode fauna of the Isle of May shags is likely dominated by *C. rudolphii*, which has been noted in astonishing numbers in other seabirds (Hoberg, 2005). *C. septentrionale* had a prevalence of only 15% in a Spanish population (Abollo et al., 2001) and has not been found in our population. *A. simplex* occurs in our population but does not reproduce in the shag as its definitive hosts are marine mammals, not birds (Anderson, 2000; H.-P. Fagerholm, pers. comm.). The identification and biology of the nematodes is treated in detail in appendix A. Although every adult shag on the Isle of May examined to date has been infected with nematodes, quantifying variation in the number of worms the host carries, i.e. nematode burden, is not straight-forward (appendix A). *In situ* measurements of worm burden using endoscopy have shown that burdens in adults are lowest in early-breeding females and highest in late-breeding males (Burthe et al., 2013). Nematode burdens commonly vary considerably between hosts across a range of systems, with larger burdens are likely to casue greater negative consequences of infection (Anderson & May, 1978; Shaw & Dobson, 1995; Tompkins et al., 2011), and by extension the effect of anti-nematode treatment may vary between individuals. To account for this would require quantifying the nematode burdens of all experimental individuals before and after treatment, which was not possible on the scale of this thesis in the shag system: I assume that all individuals are infected, and assess between-individual

variability in burdens where sufficient data is available. Shags also host biting lice, *Eidemaniella pellucida*, but these were found in conjunction with an experimental study on timing of breeding not to affect chick growth or survival (Daunt et al., 2001a).

Shags lay a modal clutch of three eggs, each three days apart (Snow, 1963; Coulson et al., 1969; Stokland & Amundsen, 1988). Incubation begins when the second egg is laid, and together with slight differences in development time of the three eggs, this results in asynchronous hatching of the brood, with the first two eggs hatching within 24 hours of each other and the third egg c.2 days later (Snow, 1963; Potts et al., 1980; Stokland & Amundsen, 1988). The last-hatched chick grows more slowly and is less likely to survive, but these effects are less marked in more productive years (Amundsen & Stokland, 1988). This suggests that last-hatched chicks in the shag may serve a resource-tracking function, allowing parents to cut their losses in poor conditions while maintaining their reproductive potential in case of sufficient resource availability (but see Amundsen & Stokland, 1988). Further, Amundsen & Stokland (1988) observe that asymmetries brought about by extreme asynchrony may be maintained by older, larger chicks obstructing their younger siblings' access to food. Thus, parental provisioning and the allocation of resources among a brood both play a role in breeding success, are variable and thus likely subject to external influences such as parasitism.

An early text on shag ecology considers nematode infection to do "no serious damage" to the host, apart from in cases of food shortage (Snow, 1960). Since then, three experimental studies have shown that parasite infections in shags do in fact play a substantial role in the birds' ecology. Treatment of adults with ivermectin, an anti-nematode drug, at a high dose reduces absolute nematode burdens (Burthe et al., 2013), and at lower doses has measurable effects on chick growth and survial (Reed et al., 2008, 2012). Treating parents before their chicks hatch negates the seasonal decline in survival of sons, which are more expensive to rear in this sexually dimorphic species, but did not affect survival of daughters (Reed et al., 2008). Moreover, treated mothers increased their foraging more as chicks grew than sham-treated controls. Treating chicks, on the other hand, increases the growth rate of last-hatched chicks but does not affect its two older siblings (Reed et al., 2012). These results suggest that conflict between parents and offspring and between siblings has a role to play in how individual shags are affected by parasitism.

This system provides three informative contrasts to many previous studies on parasitism in families using passerine hosts. Firstly, the shag is long-lived, with a breeding lifespan of up to 22 years, and may therefore be more likely to alter current reproduc-

tive investment in response to parasitism than the shorter-lived passerines (Bize et al., 2004). Secondly, the shag's brood of three chicks provides a tractable framework to investigate the role of parasitism in behavioural interactions between all individuals in the family, but intra-familial dynamics have received little attention in this species. Thirdly, for the first time in a study on parasitism in wild bird families, we investigate the impact of an endoparasite, the costs of which have already been established. Moreover, individuals can be repeatedly observed and caught throughout the breeding season and across years, enabling longitudinal studies, and behaviour can be both observed at the nest and monitored away from the colony using data logging devices.

In addition to their amenability to our questions, understanding the role of parasites in shags is of broader ecological interest. In the marine environment, seabirds are top predators and therefore keystone species, crucial to the functioning of the ecosystem (Furness & Camphuysen, 1997; Boyd et al., 2006). The impact of parasitism on a population can lead to ecosystem-scale effects, a particular risk if keystone species are affected (Clayton & Moore, 1997; Tompkins et al., 2011). This ecological role also makes them powerful indicators of the state of the marine ecosystem (Furness & Camphuysen, 1997; Piatt et al., 2007; Lewison et al., 2012). Ecological and abiotic changes that are difficult to measure directly can be made visible through their effect on prominent species such as seabirds. Using species as indicators requires an understanding of their responses to external stresses at both the individual and population level (Piatt et al., 2007). Moreover, many seabird species are in global decline as a consequence of a range of predominantly anthropogenic ecological disturbances: competition with fisheries for food, introduced land predators of nesting birds and large-scale changes to ocean conditions (Lewison et al., 2012). If parasitism is a factor driving seabird populations, understanding its effects could have implications for conservation and management. However, to date very few studies have examined the impacts of parasitic infection on the ecology, physiology or life history of seabirds, either as individuals or populations (Skorping, 1996; Hoberg, 2005).

1.5 Aims of this thesis

The overarching aim of this thesis is to bring considerations of family conflict and intra-familial interactions into the current understanding of how parasites affect individuals during reproduction, the implications of these impacts for other family members and thus the role of parasitism in the fitness of the whole family, current and

future. I use two anti-parasite treatment experiments to explore various aspects of this interplay, examining the influence of variation in extrinsic and intrinsic factors on individuals' responses to parasitism. I concentrate on parent-offspring and between-sibling interactions, addressing between-parent interactions only briefly, to maintain a focus on tractable, defined questions.

The first experiment, in chapter 2, examines how extrinsic variability and intrinsic differences between individuals interact to influence the impact of anti-parasite treatment on chicks. Neither the variable environmental conditions nor the asymmetries between siblings, both typical of wild populations, are typically accounted for in studies of how these populations are affected by parasites, and their interaction has never previously been investigated. Chapter 3 presents the core findings of my second experiment, a simultaneous manipulation of parasite burden in parents and chicks with effects measured for both parents and chicks. This is, to my knowledge, the first such family-wide investigation of the impact of parasitism in all family members on all family members. In chapter 4, I use behavioural data from this experiment to investigate whether anti-parasite treatment can alter intra-familial conflict behaviour to enable the impacts of treatment to be distributed among family members. This is the most explicit consideration to date of the behavioural impacts of parasitism on families, incorporating interactions both between parents and offspring and between siblings. In chapter 5, I investigate the longer-term consequences of anti-parasite treatment on parents in terms of overwinter foraging behaviour and subsequent breeding. No previous study has examined the persistence of effects of chick parasitism on parents into the non-breeding period, and this is also the first comparison of the implications of parent and offspring anti-parasite treatment on parents' future breeding. In addition, in appendix A, I present details of the biology of the parasite, characterize variation in worm burdens between chicks, and assess methods to quantify worm burdens, including the first use of endoscopy on chicks for this purpose.

CHAPTER 2

Parasitism in early life: environmental conditions shape intra-brood variation in impacts of parasitism

2.1 Introduction

Parasites are an integral part of any animal community and play a key role in many ecological and evolutionary processes through the costs they impose on the host (Sheldon & Verhulst, 1996; Norris & Evans, 2000; Schmid-Hempel, 2003). These costs may be particularly important to young hosts (Hudson & Dobson, 1997; Møller, 1997; Sol et al., 2003). Firstly, juveniles may have a naive or undeveloped immune system and thus be less able to defend themselves against parasite infection (Wakelin & Apanius, 1997; Sol et al., 2003). Secondly, young animals may suffer greater marginal costs of parasitism than adults as they are already bearing high energetic costs of activities such as growth and developing thermoregulation (Moe et al., 2004a,b). Lastly, the impacts of parasitism on juveniles may be long-lasting, not only affecting the immediate success of the host, as conditions experienced in early life may alter patterns of resource allocation during development with potentially long-term consequences on survival and reproductive success (Metcalfe & Monaghan, 2001; Monaghan, 2008). Parasitism

is an important component of those early-life conditions. Parasite infection of nestling birds has been shown to alter growth patterns (Bize et al., 2003; Fitze et al., 2004a,b), affect dispersal distance (Bize et al., 2005; Tschirren et al., 2007) and even impair sexual communication later in life (Bischoff et al., 2009). Through its potentially high cost and the possibility of long-term effects on host life-history traits, parasitism in juveniles could thus have far-reaching consequences on population processes.

However, we do not expect all juveniles in a population to respond in the same way to a parasite infection, just as the impacts of parasitism vary between individual adult hosts (Shaw et al., 1998). One aspect of variation between juveniles that is likely to affect the impact of parasitism, but has not received much attention, is the position of each individual in the brood's birth or hatching order. This has been particularly well studied in birds (see Hudson & Trillmich, 2007, for a review in mammals), where brood hierarchies are often established by age differences stemming from asynchronous hatching, a result of incubation beginning before the full clutch has been laid (Stenning, 1996), or from differential development rates of embryos (Cook & Monaghan, 2004). Older siblings are generally competitively dominant, obtain more food, grow faster and are more likely to survive to independence (Mock & Parker, 1997; Drummond, 2006). Asynchrony may also facilitate brood reduction in poor conditions: the younger, subordinate chicks receive less post-hatching parental investment, and are more likely to die if resources become scarce (Mock & Forbes, 1995; Forbes & Mock, 1998; Forbes, 2009). Marginal siblings may thus be in poorer overall condition and, therefore, less able to bear the costs of a parasite infection than their older siblings.

In addition to differences in age, there may be variation through the clutch in maternal allocation of substances such as hormones and immune factors which could also affect a chick's ability to deal with a parasite infection. Variation in egg testosterone, for example (Schwabl, 1993; Schwabl et al., 1997; Reed & Vleck, 2001; Poisbleau et al., 2011), may influence aggressiveness (Groothuis et al., 2005) and thus access to resources. Mothers may also alter allocation of antibodies to eggs through the clutch (Hasselquist & Nilsson, 2009), with potential implications for chicks' defences against parasitism. Last-laid eggs of collared flycatchers *Ficedula albicollis*, for example, have a higher concentration of maternal antibodies (Hargitai et al., 2006), while in zebra finches *Taeniopygia guttata*, antibody concentration in 3-day-old male embryos declines through the clutch but does not vary in female embryos (Martyka et al., 2011). Maternal antibodies have been shown to be functional (reviewed by Grindstaff et al. (2003)) and to persist for a substantial part of chick-rearing (Garnier et al., 2011).

Together, age and maternal allocation could shape an individual's exposure to parasites, its level of protection against an initial infection and its ability to cope with the costs of an established infection. However, the ultimate effect on chick fitness of this variation is not clear, as few studies of chick parasitism examine these intra-brood differences. Parasitism has been shown to impact more on certain brood members in two distinct species. In mountain bluebirds *Sialia currucoides*, experimental ectoparasite removal caused a greater change in growth rate for chicks in the middle of the brood than first or last hatchers (O'Brien & Dawson, 2009). In contrast, when chicks of the European shag *Phalacrocorax aristotelis* were treated with an anti-parasite drug, last-hatched siblings increased their growth rate to match that of their senior siblings, whose growth rate did not change (Reed et al., 2012).

In addition to intrinsic differences between siblings in the consequences of infection for individuals, parasitism may also change the way brood members interact. If this alters other siblings' exposure or responses to parasitism, such behavioural change could extend the consequences of parasitism in one chick to the whole brood. In altricial species, siblings are not independent but compete with one another for limited resources from the parents. How much food each chick gets depends both on the total amount that parents bring – provisioning – and how it is divided among the brood – allocation (as defined by Mock et al. (2011)). Provisioning and allocation can potentially be manipulated by the chicks in two ways: signals to the parents, which parents may or may not respond to (Godfray, 1995; Parker et al., 2002; Smiseth et al., 2008), and competition between siblings, which often involves outright aggression but where signalling may also be a component (Mock & Parker, 1997; Royle et al., 2002). Parasitism has indeed been shown to affect provisioning by increasing chick signalling: in great tits *Parus major*, parasitic hen fleas in the nest increase both chick begging rate and the father's nest visitation rate, a proxy for provisioning (Christe et al., 1996). However, little is known about whether parasitic infection could also alter resource allocation among a brood, which could result in differing impacts of parasitism for individual chicks even without a change in total provisioning. Reed et al. (2012) demonstrated a greater cost of parasites for the last-hatched chick and suggest, but do not test, that parasitism could exaggerate the rank hierarchy by decreasing the ability of the last-hatched chick to obtain food.

As well as varying between brood members, the costs of parasite infection are also likely to vary with environmental conditions. In unfavourable conditions, the marginal cost of parasitism may be more detrimental. This has been observed in sage thrash-

ers *Oreoscoptes montanus*, where blow fly parasitism of nestlings increased mortality only when the breeding season was cold and wet (Howe, 1992). Interactions between parasitism and environmental conditions (as indicated by food quality) has been experimentally demonstrated: in mountain bluebirds, ectoparasites reduced chick growth rate less when the parents' diet was supplemented with carotenoids (O'Brien & Dawson, 2008); in ring-necked pheasants *Phasianus colchicus*, chicks fed on a diet supplemented with antioxidants had lower parasite burdens as adults (Orledge et al., 2012); and in mealworm beetles *Tenebrio molitor*, adults infected with rat tapeworm *Hymenolepis diminuta* selected more nutrient-rich food to counteract a parasite-induced decrease in fecundity (Ponton et al., 2011). However, just as we expect individuals in a population to differ in how they are affected by parasites, individuals are expected to respond differently to a given set of external conditions (e.g. Mock & Forbes, 1995; Lewis et al., 2009). By extension, individuals may differ in how they are affected by the environment-by-parasite interaction. For example, in a less favourable breeding season, within-brood competition for food might be greater and hence later-hatched brood members may be in poorer condition than their older siblings (Mock & Forbes, 1995) and consequently more susceptible to additional costs such as parasitism than their older siblings. In a more productive year, all chicks might be in similar condition and thus be similarly affected by a given parasite burden.

In this study we investigate how hatching order and environmental conditions influence the impact of parasites on nestling European shags. Shags are infected with parasitic gastrointestinal nematodes via the fish they eat, and parasitism of chicks and adults has been shown experimentally to affect chick growth rate and survival respectively (Reed et al., 2008, 2012). Moreover, shags exhibit hatching asynchrony and hence a brood hierarchy, and a previous treatment experiment has suggested that the growth rate of last-hatched chicks is more strongly constrained by parasitism than that of their older siblings (Reed et al., 2012). In concert with a relatively fixed clutch size (3 eggs in 80% of clucthes), this indicates that shag parents adjust brood size to match environmental conditions after hatching, rather than through variation in clutch size as in other species (Winkler & Allen, 1996; Korpimaki & Wiehn, 1998, e.g.). Therefore, the difference between siblings in impacts of anti-parasite treatment has been suggested to depend on within-brood competition (Reed et al., 2012). Shag breeding success varies considerably from year to year, and in the North Sea has been shown to reflect stocks of the shags' main prey, the lesser sandeel *Ammodytes marinus*, as well as variation in weather and climate (Frederiksen et al., 2007b; Burthe et al., 2012).

This indicates that years differ in the supply of food that parents are able to provide for their chicks, and hence that within-brood competition dynamics could vary between years. If so, differences between chicks in the impacts of anti-parasite treatment may be less marked in years with more favourable conditions. Here, we explore the effect of environmental conditions on individual shag chicks' responses to endoparasitism depending on their position in the brood using experimental anti-parasite treatment across four variable breeding seasons. We predict that the impact of treatment will differ between older chicks and their last-hatched siblings, but less so in more favourable years, i.e. an interaction between treatment, hatching order and environmental conditions. We assess the relative importance of resource allocation among siblings and provisioning to the whole brood in mediating these effects by comparing individual and family-level responses. If treatment increases provisioning, some or all chicks could grow faster without depriving siblings of their share of resources. If so, we expect an effect of treatment on individual chicks and on whole-brood growth rate. On the other hand, treatment may only alter allocation of resources among siblings, such that a chick could only obtain more food at a cost to its siblings. This case would be evidenced by an effect of treatment on individual chick growth but not on the whole brood.

2.2 Methods

2.2.1 Study site & species

This study was carried out on the breeding population of shags on the Isle of May in the Firth of Forth, south-east Scotland (56° 11 N, 2° 33 W). Breeding success is very variable between years; in the last decade (2000–2010), average annual breeding success has ranged from 0.25 to 2.04 fledged chicks per nest (Newell et al., 2010) (table 2.1). The modal clutch size is three eggs (range 1–4), each laid three days apart (Snow, 1960; pers. obs.). Incubation begins when the second egg is laid, creating a hatching asynchrony: the first two eggs (the A & B chicks) hatch within 24 hours of one another, while the third egg (the C chick) hatches around 2 days later (Stokland & Amundsen, 1988; pers. obs.). This creates a size hierarchy where the C chick is frequently visibly smaller than its older siblings over half-way through chick-rearing (across the four years, at age 25 days of a 50-day nestling period, C chick 9% smaller, $p < 0.001$). Chick mortality is highest in the first 10 days after hatching (Daunt et al.,

1999) and higher for last-hatched chicks (Amundsen & Stokland, 1988). Chicks grow at a rate of 50–60g of mass a day during the linear phase of growth, from age 8 to 30 days, with males growing faster than females in this sexually dimorphic species (Daunt et al., 2001b).

In this population, 100% of adults are infected with parasitic gastrointestinal ascaridoid nematodes, predominantly *Contracaecum rudolphii* (Burthe et al., 2013, 68 adults assessed using endoscopy). Adult *C. rudolphii* in their definitive seabird host release eggs into the marine environment via the bird's faeces (Anderson, 2000; Fagerholm & Overstreet, 2008). These embryonate in water and hatch as third-stage larvae (L3) which are eaten by small crustaceans, which in turn are eaten by fish and these by the bird (Anderson, 2000; Fagerholm & Overstreet, 2008; Moravec, 2009). The worm remains in the bird's proventriculus where it moults to the reproductive adult stage and feeds on food ingested by the host (Anderson, 2000; Abollo et al., 2001; Fagerholm & Overstreet, 2008). Chicks are infected primarily by L3 worms in the fish in the regurgitated food they receive from their parents from an early age; direct transmission of adult worms dislodged from the parents' proventriculus may also occur occasionally (Hoberg, 2005; Fagerholm & Overstreet, 2008; appendix A, this thesis). Post-mortem examinations confirmed nematode infections in 100% of 31 chicks aged 12–40 days (mean burden 35 individual nematodes, range 8–148 with a high proportion (mean 85%) of larvae) in the proventriculus and lower oesophagus (S. Burthe & H. Granroth-Wilding, pers. obs.). A detailed description of the biology of the worm and details of the dissections are given in appendix A. Shags also host biting lice *Eidemanniella pellucida*, but no effect of lice on chick growth or survival has previously been detected (Daunt et al., 2001a), indicating that the impacts of anti-parasite treatment on shags are caused to a large extent by its impact on gastrointestinal nematodes. Moreover, while the drug we use (ivermectin) has traditionally been seen as a broad-spectrum anti-parasite treatment that targets arthropods as well as nematodes, recent evidence from mammalian systems suggests that the efficacy of ivermectin on ectoparasites may be low compared to its action on nematodes (Pedersen & Antonovics, 2013; Knowles et al., 2013). Nematode infections in seabirds are rarely lethal (Hoberg, 2005; Fagerholm & Overstreet, 2008) but do cause damage such as inflammation, ulceration, tissue necrosis and secondary bacterial infections at the attachment site on the bird's stomach wall (Hoberg, 2005; Abollo et al., 2001; S. Burthe & H. Granroth-Wilding, pers. obs.).

Table 2.1: A summary of the sample sizes and environmental variability in each year of the study. Productivity is measured as the number of chicks fledged per nest built across a series of long-term monitoring plots around the study colony, not used in any experiments.

Year	No. control nests	No. drug-treated nests	No. chicks with growth rates	Mean growth rate (g/day)	Colony-wide productivity (chicks/nest)
2006	18	20	109	54.4	1.22
2007	12	9	46	51.2	1.07
2010	13	23	107	54.5	2.04
2011	8	8	47	57.2	1.52

The years in which this study was carried out differed markedly in breeding success (table 2.1) measured as the average number of fledged young per built nest in a series of monitoring plots around the Isle of May, henceforth "productivity". In shags, as in other seabird species, productivity integrates several environmental factors including food, climate and weather variables (Frederiksen et al., 2004, 2007b,a) and we use it here as an annual proxy of environmental conditions.

2.2.2 Anti-parasite treatment experiment

To examine the effects of parasitism on different brood members, we treated broods of individually marked chicks with an anti-nematode drug (ivernectin) and compared these to controls in four years that differed in productivity. A broadly similar protocol was followed each breeding season (April–July) in 2006, 2007, 2010 and 2011 (table 2.1). All blood sampling and drug administration was done under UK Home Office licence (in work for this thesis, licence nos. PIL 60/12450, PPL 60/3444), all ringing under licence from the British Trust for Ornithology, and experimental procedures under a National Nature Reserve research license from Scottish Natural Heritage, the landowners at the study site (for this thesis, licence nos MON/RP/115 & MON/RP/124).

All study nests were monitored daily from the colony-wide onset of laying to obtain laying dates, which were used to predict hatching date, assuming a mean incubation period of 35 days (Potts et al., 1980). Nests were visited every 1–2 days around predicted hatching to obtain hatching date and order for each chick. Newly hatched chicks

were blood sampled (up to 50μl) for molecular sexing: DNA was extracted (DNeasy ®kit, Qiagen), a sex-linked CHD gene amplified by PCR (Griffiths et al., 1996) and the product run on a 2% agarose gel. At hatching, chicks were marked individually using coloured wool or electrical tape around the tarsus, which was changed every 2 or 5 days respectively to maintain identification. Chicks were fitted with permanent metal rings at c.15 days old.

This experiment focused on broods with three chicks still alive at the point of treatment as we expect the most pronounced rank effects between the last-hatched chick and its two older siblings, where the main age and size disparity lies. Treatment was carried out when the oldest chick in a brood was 8–14 days old, with all siblings treated simultaneously. This age range was chosen for treatment as all the chicks in the brood would be at an early stage in the linear growth phase (age 8–30 days, Daunt et al., 2001b). Treatments were assigned randomly, matching control and drug-treated nests for hatch date and colony area. Drug-treated chicks were injected subcutaneously with 0.05ml of 1% wt/vol ivermectin (Panomec©by Merial). Control broods were not treated in 2006 and sham-treated with 0.05 distilled water (2007) or saline (2010 & 2011). Previous studies have found no difference between sham-treated and unmanipulated controls in any of the dependent variables investigated (Reed et al., 2008, 2012).

At treatment, ranks were assigned according to size: the heaviest chick was ranked A, the middle chick B, and the lightest C. Mass asymmetry is likely to be a key driver of within-brood dynamics and, moreover, size at this age correctly identifies the last-hatched chick in 90% of cases (120 out of 134 nests in a subset of nests in 2010 and 2011 for which accurate hatching order was known). Average masses at treatment for the different ranks ± 1 standard error were: A chick 378 ± 11.4g, B chick 330 ± 10.5g and C chick 244 ± 11.9g. All chicks were given the same dose volume because accurate dose adjustment to these small mass differences was not possible, so averages doses were: 1.3mg/kg for A chicks, 1.5mg/kg for B chicks and 2.0mg/kg for C chicks. Younger siblings thus received a larger dose per mass, which could lead to greater effects of treatment on later-hatched chicks without any other differences between chicks being present. We address the role of this dose bias statistically in all analyses, and there is no evidence from any experimental studies on this sytem that dose differences between siblings account for wihin-brood differences in treatment impacts (Reed et al., 2012; this chapter and chapter 3, this thesis). These doses are all within an established safe range for adult shags (Burthe et al., 2013) and have previously been used on chicks with no negative consequences on survival (Reed et al., 2012).

All chicks in each nest were weighed every 4–7 days until the oldest chick was aged approximately 28–30 days, the end of the linear growth period (Daunt et al., 2001b). Chick measurements beyond the age of 30 days were not possible due to chicks becoming more mobile and an associated risk of disturbance triggering premature fledging. From these data we calculated individual growth rates by fitting a linear regression for each chick. Each chick had 3–5 measurement points (mean 4.7, 1231 measurements on 259 chicks) apart from in 2007, when only two measurements per chick (at treatment and 30 days) were possible. Reed et al. (2012) demonstrated the data to be quantitatively robust to this restricted sampling by comparing the full 2006 dataset to one restricted to two masses per chick (treatment and day 30). Throughout, weights were measured to the nearest 0.5g for chicks up to 50g, 1g up to 300g, 2.5g up to 600g, 5g up to 1000g and 10g up to 2000g. After the linear growth period, nests continued to be monitored regularly for chick survival until fledging at $\sim$50 days (Snow, 1960).

2.2.3 Efficacy of treatment

To ascertain the efficacy of treatment, we counted the number of nematode eggs in faecal samples before and after treatment in 2010. We collected faecal samples every time a chick excreted during handling and obtained samples from 60 chicks in 34 nests before treatment (29 controls, 31 treated) and from 102 chicks in 42 nests after treatment (47 controls and 55 drug-treated), of which 54 chicks in 33 nests had both before- and after-treatment samples (28 controls, 26 drug-treated). Samples were classified as pre- or post-treatment, with a spread of ages (15–25 days) in the post-treatment group. Samples were frozen and stored at -20°C. We used a flotation technique to obtain faecal egg counts as a measure of infection (adjusted from Bowman & Georgi (2009)). The sample was mixed well with concentrated salt solution at a ratio of 20ml solution for 1g of faeces and left for at least 60 seconds to allow most of the organic debris to settle. Using a pipette, the sample was then mixed gently without disturbing the layer of debris and an aliquot of 0.15ml taken while raising the pipette up through the liquid. This sample was placed in a McMaster slide and all nematode eggs under the grid, identified morphologically, counted under a light microscope at 40x magnification. This was repeated for 3 aliquots from each sample, totalling 0.0225g of faeces.

2.2.4 Statistical analysis

We assessed the impact of anti-nematode treatment on different siblings in terms of individual chick growth and survival, and on the combined growth of the brood as a whole. We expect differences in growth rate between nest-mates to reflect how resources are allocated among siblings, and whole-brood growth rate to reflect total parental provisioning. Our balanced experimental design gave a limited number of terms to be tested: treatment, rank, sex and productivity. We fitted rank as a two-level factor, comparing A & B chicks against C chick. This was partly because rank assignments at day 10 based on small mass differences between the two older chicks may not accurately reflect hatching order (whereas the C chicks is more easily identifiable), and partly because we expect rank-dependent effects of treatment to be driven by the last-hatched chick (as suggested by Reed et al. (2012)). Indeed, using rank as a three-level factor (A vs. B vs. C) gave poorer model fits (best-fit model with two-level rank, $\Delta\text{AIC} = -1.9$ compared to three-level rank).

2.2.4.1 Individual growth rates

First, we examined how anti-nematode treatment interplays with rank, sex and productivity to affect individual chick growth rate. Rank and treatment have previously been shown to interact (Reed et al., 2012), and we examined whether this varies with environmental conditions by testing a three-way interaction with productivity. All models included sex to account for the faster growth of males. The simplest model to address our key question was therefore:

lme: Growth rate $\sim$ Rank $*$ Treatment $*$ Productivity $+$ Sex ($+$ Nest as random)

To examine whether effects of rank are simply a result of mass differences at treatment between older and younger siblings, we also fitted mass at treatment instead of rank:

lme: Growth rate $\sim$ Mass at treatment $*$ Treatment $*$ Productivity $+$ Sex ($+$ Nest)

Because faster-growing sons may be more expensive to rear (Reed et al., 2008), we also tested whether male and female chicks responded differently to treatment, productivity or their position in the brood.

2.2.4.2 Whole-brood growth rates

Secondly, we modelled whole-brood growth rate. In addition to treatment and productivity, these models also included brood size (for the majority of the experimental

period), to account for nests in which chicks died after treatment, and brood sex ratio (number of males divided by brood size), as more chicks and more males would necessarily lead to faster whole-brood growth. The full model was therefore:

$$\text{lm: Growth rate} \sim \text{Treatment} * \text{Productivity} + \text{Brood size} * \text{Sex ratio}$$

2.2.4.3 Chick survival

Finally, we investigated whether treatment affected chick survival after treatment to fledging. We applied the models described for chick growth above to individual survival from treatment to fledging. Survival was modelled as a binary response with nest as a random factor. The full survival model was therefore:

$$\text{glmm: Survival} \sim \text{Rank} * \text{Treatment} * \text{Productivity} + \text{Sex} (+ \text{Nest})$$

2.2.4.4 Statistical modelling

We selected best-fit models using AIC. For each response variable, we tested the importance of the predicted interactions against more parsimonious explanations by comparing the model including the predicted interaction against models including all of its lower-level interactions and main effects, either additively or singly.

In analysing the faecal egg counts, we fit a negative binomial model to account for the heavy skew in egg counts (many zeros and few very high counts) common in parasitological data (Shaw et al., 1998). To avoid pseudo-replication caused by partial resampling of chicks, we analysed pre- and post-dose samples separately. We examined whether ranks differed in their worm burdens at the start of the experiment by testing the effect of rank on initial egg count, and examined whether rank and treatment interacted to affect post-treatment egg count as well as growth rate.

All analyses were conducted in R version 2.13.1 (R Development Core Team, 2011). Chick growth rate was modelled using linear mixed models (LMMs) in the package nlme (Pinheiro et al., 2012), fitting nest as the random factor. Whole-brood growth rate required no random factors and was modelled using simple linear models in the package stats (R Development Core Team, 2011). Chick survival was modelled using generalized linear mixed models (GLMMs) with a binary response variable (binomial errors, logit link) with nest as a random factor in the package lme4 (Bates et al., 2011), and egg counts in a negative binomial GLM in the package MASS (Venables & Ripley, 2002). All parameter estimates and effect sizes are presented as mean$\pm$1 standard error.

2.3 Results

2.3.1 Efficacy of treatment

Before treatment, drug-treated and control shag chicks had similar numbers of nematode eggs in their faeces (-0.2 ± 0.9, $z = -0.17$, $p = 0.865$) (fig. 2.1). At 5–15 days after treatment, egg counts were significantly lower in drug-treated chicks than in control chicks (-3.7 ± 0.9; $z = -4.20$, $p < 0.001$; fig. 2.1).

Chicks of all ranks had similar faecal egg counts both before treatment (for both B and C chicks compared to A, $p > 0.4$) and after (rank-by treatment interaction for both B and C chicks compared to A, $p > 0.8$; main effect of rank in addition to treatment, both $p > 0.2$).

2.3.2 Individual growth rates

We examined the effect of anti-nematode treatment on the growth of different brood members in 111 nests (309 chicks that survived to fledging, i.e. with growth rates) across four years with variable productivity (the best year had almost twice the productivity of the worst, table 2.1). Chicks of different rank responded differently to treatment, but this varied with productivity (table 2.2; fig. 2.2). Neither productivity nor treatment affected the growth of older siblings whereas C chicks responded to both factors. Among control chicks (natural levels of parasite infection), C chicks grew faster in more productive years. Anti-parasite treatment altered C chick growth rate, but the direction of this effect depended on productivity: treatment increased C chick growth rate in less productive years and decreased it in the most productive year. As a result, in treated broods, all chicks responded similarly to productivity (fig. 2.2). The effect of rank is not simply a consequence of size differences at treatment: if the same model is fitted to mass at treatment instead of rank, the three-way interaction is not significant ($F_{1,174} = 1.11$, $p = 0.247$).

Overall, males grew faster than females (56.1 ± 0.4 g/day compared to 52.8 ± 0.4 g/day) and growth rate was lower for younger siblings (A chick 55.9 ± 0.4, B chick 54.2 ± 0.5, C chick 52.9 ± 0.6). Sexes were evenly distributed through the clutch (chi-squared on sex and blocked ranks, $\chi^2 = 0.98$, d.f.$= 1$, $p = 0.322$).

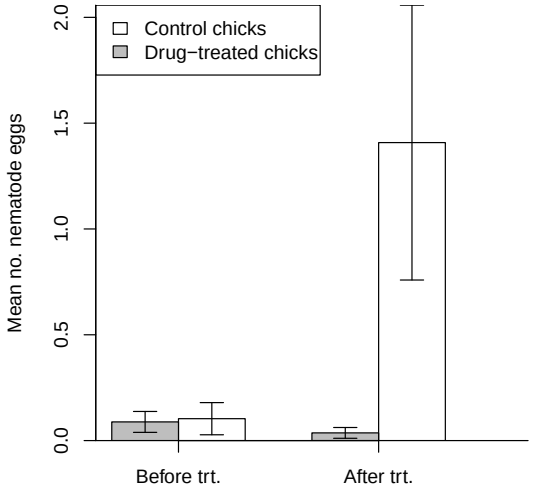


Figure 2.1: The number of nematode eggs in shag chick faecal samples before and after treatment for control chicks (white bars) and anti-nematode treated chicks (grey bars). Bars show mean egg counts ± 1 standard error.

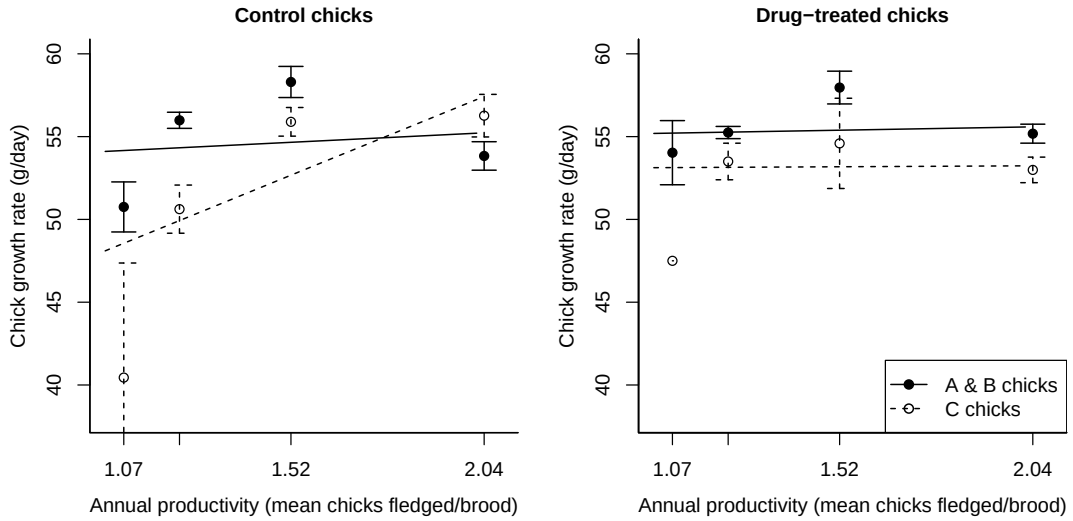


Figure 2.2: The growth rate of siblings of different ranks in control broods (left panel) and in anti-nematode treated broods (right panel) across a range of environmental conditions, represented by annual productivity. A chicks are shown by the black solid line and filled symbols, B chicks by the black long-hatched line with open symbols, and C chicks by the blue semi-hatched line with filled symbols. The plot shows mean values ± 1 standard error for each rank in each year, and the lines show the fitted relationship.

Table 2.2: Comparison of all models (including biologically meaningful interactions) tested to describe chick growth rate and parameter estimates for the best-fit model. ΔAICs are in relation to the best fit model, and models are presented in order of decreasing fit.

Model	**ΔAIC**
Rank * Treatment * Productivity + Sex * Rank	0.0
Rank * Treatment * Productivity + Sex * Rank * Treatment	1.8
Rank * Treatment * Productivity + Sex	2.2
Sex * Rank + Rank * Productivity	3.3
Rank * Productivity + Sex	6.0
Rank * Treatment + Rank * Productivity + Sex * Rank	6.2
Rank * Sex + Productivity	7.6
Sex * Rank * Treatment + Rank * Productivity	8.0
Rank + Sex + Productivity + Treatment	11.4

***Best fit model*:**

Term	**Estimate**	**Std. error**	**d.f.**	**t-value**	**p-value**
Rank $*$ Treatment $*$ Productivity	-7.1	2.7	192	-2.65	0.009
Rank $*$ Sex	2.3	1.1	192	2.02	0.044

2.3.3 Whole-brood growth rates

The combined growth rate of the whole brood was not affected by treatment, either overall or depending on productivity (fig. 2.3, table 2.3). All broods grew more slowly in less productive years. As expected, brood size and sex ratio both affected whole-brood growth rate, including their interaction, such that a higher proportion of males increased whole-brood growth rate more in broods that were smaller for the majority of chick-rearing (model of these main effects and their interaction: interaction, $F_{1,107} = 7.73$, $p = 0.006$; main effects both $p < 0.002$).

2.3.4 Survival

Anti-parasite treatment did not significantly affect survival from treatment to fledging in any year or for any rank (table 2.4), although the best fit model included a rank * treatment interaction in which drug-treatment reduced survival in C chicks but not

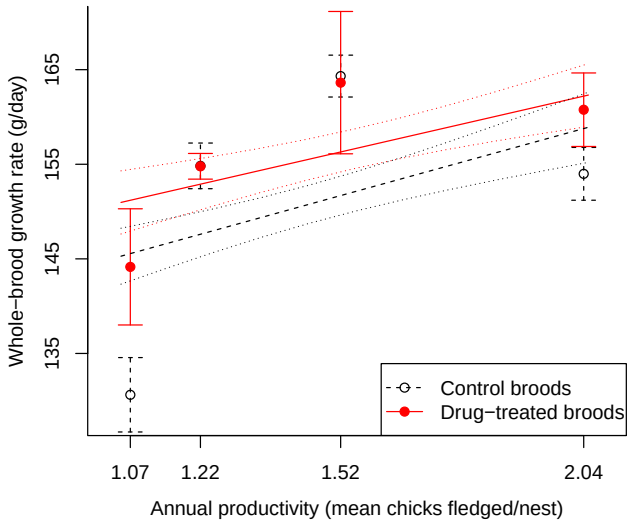


Figure 2.3: The combined growth rate for the whole brood in relation to environmental conditions (productivity) for anti-nematode treated and control broods. Control broods are shown in black and drug-treated broods in red. Points show mean values and lines the fitted relationship, both ± 1 standard error. The y-axis shows growth rate as the residual from a significant brood size $*$ sex ratio interaction, with a residual of 0 shown for clarity as the overall mean of 154 g/day.

Table 2.3: Models tested to describe the growth rate of the brood as a whole, with parameter estimates for the best fit model. ΔAICs are in relation to the best fit model, and models are presented in order of decreasing fit.

Model	**ΔAIC**
Treatment + Productivity + Sex ratio * Brood size	0.0
Treatment * Productivity + Sex ratio * Brood size	1.9
Treatment + Sex ratio * Brood size	9.4
Sex ratio * Brood size	11.1

***Best fit model*:**

Term	**Estimate**	**Std. error**	**t-value**	**p-value**
Treatment	4.6	3.0	1.52	0.131
Productivity	13.0	3.8	3.38	0.001
Sex ratio * brood size	-30.9	12.9	-2.39	0.018

Table 2.4: Models tested to investigate the effect of anti-nematode treatment on chick survival and parameter estimates for the best fit model. Productivity is shortened to "Prod." for space. ΔAICs are shown relative to the model including only main effects. Sex did not affect survival and is not shown here (in addition to rank and productivity, sex main effect and all interactions, $\Delta\text{AIC} > -0.3$).

Model	**ΔAIC**	**AIC weight**		
Rank * Treatment + Prod.	0			
Prod. * Treatment + Rank	1.3			
Rank + Prod. + Treatment	2.6			
Rank * Prod. + Treatment	3.3			
Rank * Treatment * Prod.	3.5			
Best fit model:				
Parameter	**Estimate**	**Std. error**	**z-value**	**p-value**
Productivity	2.4	0.8	3.20	0.001
Rank (C chick effect)	−2.3	0.6	−3.64	0.000
Treatment	1.9	1.4	1.38	0.169
Rank * Treatment	−2.4	1.4	−1.65	0.099

in A & B chicks. C chicks were less likely to fledge irrespective of treatment (main effect of rank, $p < 0.001$) and all brood members had greater fledging success in more productive years (main effect of productivity, $p = 0.002$). Sex did not affect fledging success (main effect and all interactions, $p > 0.1$, $\Delta\text{AIC} > -0.3$).

2.4 Discussion

In this study, we have shown experimentally that broad environmental conditions influence the way in which nestling shags respond to anti-nematode treatment, and that this influence differs between siblings. In control broods, last-hatched chicks grew faster in more productive years, but anti-nematode treatment removed this sensitivity to environmental conditions. In contrast, the older two siblings grew at similar rates in all four study years and irrespective of treatment. Despite these rank differences in the impact of treatment, we found no evidence that parasite burden at the beginning of the experiment differed between chicks in a nest. Treatment did not affect chick

survival, nor the growth rate of the brood as a whole. We propose two non-exclusive hypotheses for how the interaction between treatment, rank and environmental conditions could be mediated. Firstly, hatching order could be associated with intrinsic differences between siblings that influence how they are affected by parasite infection, for example in size or pre-hatching maternal allocation of resources. Secondly, chicks of different ranks in the brood hierarchy could be affected differently if parasitism alters behavioural interactions within the family to affect how much food chicks obtain.

At hatching, asynchrony can lead to difference in size and hence competitive ability among siblings (Mock & Forbes, 1995), and we have shown here for shags that last-hatched chicks grow more slowly than their older siblings in all but the most productive years. Across vertebrates, older chicks tend to be competitively dominant over their younger siblings (Drummond, 2006), which may increase differences between siblings in condition (Bonabeau et al., 1998) and hence the impacts of parasitism. We might predict that older, larger chicks would generally suffer lower marginal costs of a given parasite burden. In general, age asymmetries within a brood are considered to be adaptive, with later-hatched chicks marginal to the older, core chicks (Mock & Forbes, 1995; Forbes, 2009). These marginal offspring may serve a "resource-tracking" function, receiving less post-hatching parental investment and thus being more likely to die in poor conditions. The cost of the misplaced investment is minimized and parents are left to focus limited resources on core offspring in the reduced brood (Mock & Forbes, 1995; Bonabeau et al., 1998; Forbes et al., 2002; Forbes, 2009). However, the possibility that parasites could play a role in brood reduction is rarely considered. In shags, C chicks generally grow more slowly and suffer higher mortality than their older siblings, suggesting that they may serve at least partly as resource-tracking offspring (Amundsen & Stokland, 1988; Forbes et al., 2002; this study). Our results suggest that parasitism may contribute to the proximate causes of brood reduction by imposing greater costs on marginal chicks. Siblings appeared not to differ in their initial nematode burden, as our faecal egg counts showed, suggesting that the difference between siblings in the impacts of anti-nematode treatment is not driven by within-brood differences in infection prevalence or intensity. Instead, C chicks appear to suffer more detrimental effects of a given burden, particularly in poor conditions, although the reasons for this greater susceptibilty are as yet unclear. This hypothesis is built on our growth rate results and is only partly supported by our survival results: C chicks had higher mortality than their older siblings, and all siblings had higher mortality in poorer years, but there was no effect of treatment. However, we had little power to detect survival

effects of treatment as most chick mortality had happened by the age at which chicks were treated (Daunt et al., 1999) and, moreover, productivity was relatively high in all our study years (compare to the worst year on record for this population, 0.18 chicks fledged per nest (Harris & Wanless, 1993). Thus, similar patterns to those we show in growth rates might be seen in survival earlier in chick development or in poorer years.

The exceptionally high productivity in 2010 provided another insight into the role of the C chick in shags. In that year, control C chicks grew faster than their older siblings, suggesting a further, little-explored dimension to brood reduction: that the success of later-hatched chicks tracks resource availability not only by dying early or growing slowly in poor years, but also by increasing their growth rate to take greater advantage of the most favourable conditions. Fast growth is correlated with high fledging mass in the shag (Reed et al., 2012), and in a range of bird species high fledging mass is associated with improved recruitment probability (Magrath, 1991; Schwagmeyer & Mock, 2008) and thus increased inclusive fitness for the parents. Indeed, in lesser black-backed gulls *Larus fuscus*, marginal chicks have been shown to increase their growth rate and catch up from their initial handicap, but only late in development once survival is more or less assured (Royle, 2000). Last-hatched chicks could thus be more plastic in their growth and success in both good and bad conditions, allowing their parents to guard against missed opportunities as well as against the more commonly considered risk of over-spending. This high-risk, high-return strategy is applied by Forbes (2009), but we are not aware of any experimental studies that investigate this possibility. It should be noted, however, that faster growth rate may not always be beneficial (see discussion in chapter 6); both retarded and accelerated growth have been shown to impair various aspects of fitness (reviewed in Monaghan, 2008). Moreover, fledging mass in shags may not directly reflect the linear growth rate, but we were not able to test this relationship because it was unsafe to approach nests after the linear growth phase.

Even before hatching, certain members of a brood could be intrinsically predisposed to cope better with a parasite infection. In many species, maternal allocation of substances such as antibodies and hormones varies through a clutch (Hasselquist & Nilsson, 2009; Groothuis et al., 2005). Maternal antibodies can persist well into chick development (Garnier et al., 2011), so may still have been circulating in chicks during our experimental period, and have been shown to reduce the life span of macroparasites (Gallizzi & Richner, 2008). Similarly, maternal androgens may affect begging or aggression (Schwabl et al., 1997; Groothuis et al., 2005), influencing the amount of food

a chick obtains and thus its resources for dealing with a parasite infection. However, how maternal investment might differ between siblings in the shag is not known, and we therefore focus on the distribution of food among siblings as the likely proximate cause of treatment-induced growth differences across the brood.

The complex differences between siblings and between years in the impact of treatment suggests that behavioural interactions within the family may contribute to chicks' responses both to treatment and prevailing environmental conditions. Anti-parasite treatment could have altered the intra-familial dynamics to affect both the total amount that parents bring to the nest (provisioning) and how this is distributed between chicks (allocation). Resulting changes to how much food each chick obtains could lead to impacts of treatment beyond what we might expect from the direct costs of parasitism on individual chicks. In our study, anti-nematode treatment did not change the total growth rate of the brood, suggesting that parental provisioning to broods with natual infection prevalence was not reduced as we would expect from the patterns in individual chick growth rates. Differences in growth rate between siblings in control and anti-nematode treated broods are thus likely to be due, to a large extent, to differences in how limited food is allocated among the siblings. For example, A and B chicks may be able to maintain their competitive dominance irrespective of environmental conditions or parasitism, with the C chick's variability in growth as a passive consequence. Indeed, a cross-fostering experiment between two species of boobies (Sulidae, relatives of the Phalcrocoracidae) showed that last-hatched chicks were unable to modulate their competitive behaviour (Drummond et al., 2003). Alternatively, parents may use provisioning rules that prioritize core young up to a certain viability threshold, with surplus food given to the last-hatched chick, but we have found no evidence for such a pattern (chapter 4). We explore behavioural interactions between family members in detail in chapter 4.

In summary, we have demonstrated that environmental conditions are instrumental in shaping the response of juvenile hosts to gastrointestinal nematode infections, and that the details of that shaping differ between siblings in a brood. To our knowledge, no previous study has quantitatively demonstrated how the effect of anti-parasite treatment might vary across a natural range of external conditions. Many authors assume a general rule of greater negative consequences of parasites in worse conditions (Hoberg, 2005; Fagerholm & Overstreet, 2008), or find such a relationship between two breeding seasons (Knowles et al., 2010b) or between two experimental manipulations of environmental conditions (O'Brien & Dawson, 2008). Our study supports this

general rule, but adds two caveats: the rule may only apply to certain individuals (in our case last-hatched chicks) and, as a consequence, the relative impact of parasites on different individuals can differ with external conditions. This environment-dependence highlights the importance of repeating field experiments under different environmental conditions to fully understand observed patterns, and also raises the important question of how parasite prevalence varies with environmental conditions. The factors that affect shag breeding success may also be linked to the abundance of the nematodes' intermediate hosts (Anderson, 2000; Arnott & Ruxton, 2002; Frederiksen et al., 2007a,b; Moravec, 2009), and information on environmental and temporal patterns in parasite burdens in both intermediate and definitive hosts would be extremely valuable to understand our observed inter-annual differences. Nonetheless, we have shown that endoparasites can be an important factor in shaping juvenile development in the shag, with subtle but important variation in impacts across individuals and years that must be considered when interpreting the potential consequences of infection for population processes.

CHAPTER 3

Impacts of parasitism in adult and juvenile European shags *Phalacrocorax aristotelis* extend to other family members

3.1 Introduction

Parasite infection can have negative impacts on many aspects of host physiology (Clayton & Moore, 1997; Hudson et al., 2002). If these direct costs for the host affect its behaviour and thus its interactions with other individuals, parasitism could also indirectly affect individuals other than the host. In altricial species, dependent offspring and caring parents interact closely during the breeding season, and parasite infection thus has the potential to affect two generations simultaneously. The cost of an infection in parents could, for example, reduce their ability to provision their offspring (Råberg et al., 2000). Offspring would then pay the cost of parasitism in their parents, even if unparasitized themselves, through a resulting decrease in fitness. Similarly, an infection in offspring may increase their need for resources (O'Brien & Dawson, 2008), with a resulting change to chicks' demands on their parents and associated signalling (e.g. begging behaviour, Christe et al., 1996). If parents increase provisioning in response (Christe et al., 1996; Moreno-Rueda & Redondo, 2012), the extra resources may re-

duce the cost of the infection to the chicks but increase it for the parents, even though they themselves are not infected. Thus, part or all of the costs of parasitism in an individual could be passed to other family members, giving rise to cross-generational impacts of infection, such that offspring fitness would depend on parents' parasite burden and vice versa. While several studies have described instances of parasite infection in parents impacting on offspring and vice versa in systems including passerines, ungulates and seabirds (e.g. Christe et al., 1996; Stien et al., 2002; O'Brien & Dawson, 2009; Reed et al., 2012), the family-wide distribution of costs has not, to our knowledge, previously been investigated comprehensively in a single study. This redistribution within a family of the potential fitness consequences of parasitism can be seen as part of the evolutionary conflict among family members: between parents, between parents and offspring and between siblings. This conflict is commonly viewed from a perspective of distribution of a given amount of resources (Godfray, 1995; Mock & Parker, 1997; Parker et al., 2002; Royle et al., 2012), but it is rarely considered that witholding resources could allow family members to pass external costs onto others.

Not all family members are affected equally by parasites, and just as the direct costs of parasitism may vary between parents or siblings (e.g. Reed et al., 2008; Knowles et al., 2010a; Reed et al., 2012; chapter 2), so family members might differ in their susceptibility to cross-generational effects of parasitism. Firstly, parents may differ in how parasitism in their chicks impacts on their performance. Chick parasite burden might affect mothers and fathers differently, for example, if they differ in their responsiveness to chick signals. Mother canaries *Serinus canaria* base their provisioning decisions on both chick posture and begging intensity, while fathers only respond to posture (Kilner, 2002), and in Manx shearwaters *Puffinus puffinus*, chick calls only affect mothers' provisioning effort, not fathers' (Quillfeldt et al., 2004). Hence, if chick parasite burden modulates any source of information that parents use in feeding decisions, the more responsive parent may be more affected by parasitism in its offspring. Secondly, siblings in a brood may differ in the extent to which they are affected by parasitism in their parents. In sexually dimorphic species, for example, the more expensive sex may be more vulnerable to negative effects of parasitism in their parents: nematode infections in parent European shags *Phalacrocorax aristotelis* are associated with higher mortality of faster-growing sons but not daughters (Reed et al., 2008).

Within a brood, siblings may differ not only in their susceptibility to cross-generational costs of parasitism, but also in their value to the parents. For example, offspring of the more expensive sex may be less valuable than their cheaper siblings

if there are insufficient resources to raise them successfully (Trivers & Willard, 1973). Similarly, in species where younger siblings serve a resource-tracking function, we expect these marginal offspring to be less valuable than their core siblings (Mock & Forbes, 1995; Forbes, 2009). In addition to variation in value between offspring, parents may differ in how they value the whole brood. For example, in species with extra-pair paternity, the social father may be less confident than the mother in his genetic investment in the brood (Parker & Birkhead, 2013), which is associated with stronger conflicts of interest between family members (Hamilton, 1964b). The cross-generational impact of parasitism on both chicks and parents could thus depend on variation both in intrinsic vulnerability to these costs and in the value of chicks to parents.

Any family member could experience a combination of direct and indirect effects of parasitism, which could have additive or interacting effects on fitness. Indeed, studies in many systems have observed parasitic infection in an individual impacting on both the host itself and on other family members (Christe et al., 1996; Stien et al., 2002; Bize et al., 2004; Gallizzi et al., 2008a). However, identifying direct and indirect effects, and disentangling their relative importance, may not be straightforward if the impact of parasitism is correlated among family members. We expect such covariance for two reasons. Firstly, parasite burdens may be similar across a family. The biotic environments of family members often overlap substantially, particularly in altricial species, so they may face similar parasite populations and infection rates. In addition, one generation may act as a infection source, such that parents with a high parasite burden transmit more to their offspring, or chicks to parents (Møller, 1994; Bize et al., 2004). Secondly, family members may be similarly susceptible to the costs of a given parasite burden. Siblings and parents could have comparable levels of defence against parasites because of their shared genetic background: heritable components of humoral immunity have been demonstrated in blue tits *Cyanistes caeruleus* and domestic chickens (Råberg et al., 2003; Wijga et al., 2009), although the heritability of cell-mediated immunity in wild bird populations is less clear (Christe et al., 2000; Tella et al., 2000). On the other hand, these positive correlations may be modified by maternal transfer of antibodies: mothers with a high parasite burden have been shown to have higher levels of circulating parasite-specific antibodies and transfer more of this defence to the offspring, potentially reducing the impact of parasites for the offspring (Buechler et al., 2002; Gallizzi & Richner, 2008).

Therefore, in order to examine the distribution of the costs of parasitism among a family, within-family correlations in parasite burden or susceptibility must be controlled for experimentally. In this study, we manipulate parasite burdens of both chick and adult European shags to examine the consequences of parasitism for other family members. Previous experiments in this system have demonstrated that parasitism in adults affects mothers more heavily than fathers and sons more heavily than daughters (Reed et al., 2008), and that parasitism in chicks impacts more strongly on the last-hatched chick than on its older siblings (Reed et al., 2012; chapter 2, this thesis). This system thus displays both within-family variation in the impacts of parasitism and cross-generational transfer of fitness consequences of parasitism. Moreover, shags have a relatively high rate of extra-pair paternity (17%, Graves et al., 1993), so we expect conflict between family members over the distribution of parasite costs. However, given that the impacts of parasitism can differ between years (chapter 2), these independent parent and chick manipulation studies carried out in different years do not fully illustrate how costs of parasitism are partitioned among a family. Here, we treat parents and/or chicks with an antihelminthic drug in a two-by-two treatment design and compare them to sham-treated controls in a single breeding season. This design breaks any parent–offspring correlation in parasite burden, allowing us to examine more specifically the family-wide impacts of infection in both generations and how they might interact. We examine the distribution of family-wide direct and indirect impacts of parasitism by measuring the responses of all family members to all treatments. To our knowledge, this is the first study to explicitly examine how costs of parasitism are distributed across the whole family. We predict that anti-parasite treatment will impact not only directly on the treated generation but also indirectly on other family members, and that the relative strengths of direct and indirect effects will vary between the generations, with the direction of this balance dependent on the generations' life-history investment priorities and their physical control of intra-familial interactions. Similarly, we expect that both direct and indirect impacts of treatment will vary through the season, between the sexes, and between chicks according to hatching order.

3.2 Methods

3.2.1 Study system

This study was conducted on the breeding population of shags on the Isle of May in south-east Scotland (56° 11 N, 2° 33 W) in 2011. In this population, 100% of adults and 100% of chicks over 10 days old (68 adults endoscopes and 33 chicks dissected) are infected with gastrointestinal nematodes (Burthe et al., 2013; appendix A, this thesis), and hosting worms has been demonstrated to be costly for both parents and offspring (Reed et al., 2008, 2012; this thesis). Shags lay a modal clutch size of three eggs and incubation begins when the second egg is laid, creating a hatching asynchrony (Snow, 1960; Stokland & Amundsen, 1988). Chick mortality is highest in the first 10 days after hatching (Daunt et al., 1999) and for last-hatched chicks (chapter 2). In 2011, each nest produced an average of 1.52 chicks, which is above the long-term average of $\sim$1 (Newell et al., 2011) so the marginal costs of parasitism may be relatively low (see chapter 2). Male shags are 22% heavier than females as adults (Daunt et al., 2001b), growing at a faster rate during the linear phase of growth which occurs between the ages of 8 and 30 days (Daunt et al., 2001b).

3.2.2 Anti-parasite treatment experiment

To examine the direct and indirect impacts of parasitism for all family members while accounting for potential correlations between parent and chick nematode burden, we manipulated the nematode burden of both parents and/or all their chicks using an antihelminthic drug and compared these to sham-treated controls. The two-by-two factorial design gave four treatment groups: control parents with control chicks; control parents with drug-treated chicks; drug-treated parents with control chicks; and drug-treated parents with drug-treated chicks.

Experimental nests were monitored daily, beginning from the colony-wide onset of laying. Lay date for each nest was determined to within 2 days by observation of eggs and incubation behaviour. Nests could not be visited to determine exact laying dates as shags are sensitive to disturbance at this stage of the breeding attempt. Only three-egg nests were used in the experiment and were assigned to one of the four treatment groups matched for colony area and lay date. At 3–7 days before predicted hatching, based on an incubation period of 35 days (Potts et al., 1980), both parents on a nest

were caught and weighed and their head-bill length measured. They were then injected intramuscularly with either a saline control or the broad-spectrum wormer ivermectin (Panomec©by Merial, 1% wt/vol) at a dose of 0.7mg/kg. Both members of a pair were given the same treatment.

From 3 days before predicted hatching, nests were visited daily to obtain accurate hatching dates and hatching order within a brood. Pipping chicks were marked individually with coloured pen on the egg tooth. Newly hatched chicks were blood sampled for molecular sexing (Griffiths et al., 1996) and marked individually using tabs of electrical tape that expanded as the chick grew and were checked regularly. Ranks were assigned according to hatching order: the first-hatched chick was ranked A, the second B and third C. When the oldest chick was 10–12 days old (henceforth "day 10–12"), all chicks in the brood were weighed and injected subcutaneously with 0.05ml of either ivermectin or a saline control, with chick treatments matched for parent treatment and brood size in addition to hatch date and colony area. Differences in mass at treatment between siblings were too small to allow meaningful dose adjustments, so average dose varied through the brood (A chicks mean 1.6mg/kg, B chicks 1.9mg/kg, C chicks 2.4mg/kg), but we have previously shown in several years that chick responses to treatment depend on hatching order, not mass at treatment (Reed et al., 2012; chapter 2). In this study, chicks that were heavier at treatment grew faster (as main effect in addition to sex, effect of mass at treatment on growth rate $+0.02 \pm 0.00$g/day, $p < 0.001$), but mass at treatment did not affect the impact of treatment (treatment mass * chick treatment and treatment mass * adult treatment both $p > 0.4$). On day 15, 22, 28 and 35 (all ± 1 day) the chicks were weighed again, to the nearest 1g up to 300g, 2.5g up to 600g, 5g up to 1000g and 10g up to 2000g. Chicks were given permanent metal rings when large enough at c.15 days old. At the end of the experimental period, when chicks were 30–35 days old, adults were caught and weighed again. Adults were caught throughout the day, but time of weighing did not affect mass (in this study, linear model of mass predicted by head-bill length and time: head-bill, $p < 0.001$; time, $p = 0.163$).

All blood sampling and injection was carried out under Home Office licence (personal licence no. PIL 60/12450, project licence PPL 60/3444), ringing under license from the British Trust for Ornithology, and experiments under a National Nature Reserve research licence from Scottish Natural Heritage (license no. MON/RP/124).

3.2.3 Statistical analysis

We considered the effects of both parent and chick treatments on all family members (parent mass change, chick survival and chick growth; details in next section). In all response variables, we tested for effects of both treatments as main effects and their interaction. We also investigated whether any treatment effects varied between individuals, testing both treatments in simple interactions with hatch date, parent sex, chick sex and chick rank, all of which have previously been shown to affect individuals' responses to treatment (Reed et al., 2008, 2012; chapter 2). Chicks of different sex and rank were evenly distributed through the treatment groups (χ^2-test on parent treatment, chick treatment & sex: $\chi^2 = 3.69$, d.f.$= 7$, $p = 0.815$; on parent treatment, chick treatment & rank: $\chi^2 = 7.04$, d.f.$= 11$, $p = 0.796$). Further, we examined whether differences between individuals in treatment response were exacerbated later in the season, as the Isle of May shags display pronounced seasonal declines in success (e.g. Daunt et al., 1999; Reed et al., 2008). We therefore tested three-way interactions with treatment including sex * hatch date and rank * hatch date.

We measured treatment effects on parents as absolute change in mass between the start and the end of the experimental period. Treatment effects on mass change in chicks was measured as growth rate, obtained by fitting a linear regression through the four masses during the linear growth phase for each chick. This accounted for variation in chick masses due to recently received food, which may be of considerable mass (see chapter 4) but does not directly reflect condition. We also examined the effects of treatment on chick survival, modelled as a binary response with binomial errors and a logit link. We used three measures of survival and derived separate background models for each one: survival from the point of parent treatment (before hatching), survival from hatching (henceforth post-hatching survival), and survival from age 10 days, when chick treatment took place. Survival from parent treatment allowed us to test for combined treatment effects on hatching success and post-hatching survival; out of 189 eggs at the start of the experiment, 22 did not hatch. However, to examine the effects of chick sex and rank we could only use post-hatching survival, as these attributes were only determined at hatching. Treatment could affect survival differently for chicks of different sex, as more expensive sons could have different mortality to daughters, and rank, as C chicks have higher mortality. For all response variables, all models of treatment effects included terms to account for natural, non-experimental variation in all response variables measured (table 3.1; details of testing for non-experimental sources

Table 3.1: Significant non-experimental sources of variation in all response variables tested. All models of treatment effects included these terms. For interactions with rank, the statistics presented are for C chicks compared to A chicks; in no cases did B chicks differ significantly from A chicks (all $p > 0.1$). For interactions with sex, the statistics are for males compared to females. The parental mass change effect is measured in grams over the experimental period, and chick growth rate as g/day. For survival models, the effect size is not back-transformed from the logit link function used for these binary response variables. Full model derivations are presented in appendix B.

Model (Term)	**Parameter estimate**	**p-value**
Mass change $\sim$ Sex $*$ Hatch date	-7.72 ± 3.37	0.026
Growth rate $\sim$ Sex $+$ Rank		
Sex	$+3.27 \pm 0.55$	<0.001
Rank	-1.92 ± 0.66	0.0048
Survival from egg $\sim$ Hatch date	-0.08 ± 0.03	0.003
Post-hatching survival $\sim$ Rank $*$ Hatch date	-0.35 ± 0.17	0.024
Post-treatment survival $\sim$ Hatch date $+$ Rank		
Hatch date	-0.17 ± 0.05	0.001
Rank	-3.44 ± 1.23	0.005

variation in appendix B).

We further examined the role of hatch date by testing whether effects of phenology on responses to treatment were driven by differences in parental age (known exactly for 63 out of our 106 parents, with a minimum age for the remaining 43) as older birds tend to breed earlier in the season (Daunt et al., 1999). We found no effects of age per se in either natural variation or treatment effects in any response, nor did parental age explain any treatment effects better than hatch date (details in appendix B). We also investigated whether phenological trends were explained by later-breeding parents being in worse condition (mass / head-bill^3) at initial capture, but found no such effect for mothers or fathers (effect of hatch date, in addition to sex, on condition at initial capture: $< 0.01 \pm 0.01$, $p = 0.944$; sex * hatch date on condition: 0.02 ± 0.02, $p = 0.305$).

All analysis was done in R 2.15.1 (R Development Core Team, 2011) using the packages nlme (Pinheiro et al., 2012) and lme4 (Bates et al., 2011). All responses were

Table 3.2: Sample sizes, divided by each treatment group, used in the analysis. All nests had three eggs at the start of the experiment. Parents of failed nests left the nest site and could not be recaught to measure mass change.

Chick treatment	**Parent treatment**	
	Control	Drug-treated
Control	17 nests 36 chicks, 31 adults	15 nests 34 chicks, 26 adults
Drug-treated	14 nests 32 chicks, 23 adults	14 nests 32 chicks, 26 adults
Failed before treatment	1 nest 0 chicks or adults	2 nests 0 chicks or adults

modelled using (generalized) linear mixed effects models, with nest fitted as a random factor to account for non-independence of siblings and of parent pairs. In total, we manipulated 71 nests in treatment groups as shown in table 3.2. We excluded one nest from the analysis where only one parent could be caught for ivermectin treatment, but retained two nests where only one parent could be caught for control treatment as a previous adult dosing study found no difference between unmanipulated and sham-treated controls (Reed et al., 2008). Excluding these nests made no qualitative difference to our main results. Four nests were excluded because they were discovered to be second clutches after an earlier failure or because the adult pair were related. We also excluded three nests with hatch dates $>$ 10 days after the latest nest in the main hatch date distribution (range 31 days). These late nests had strong leverage on the results, altering the outcome of some analyses in a way that misrepresented the responses to treatment in the majority of the experimental nests. Our analysis therefore used nests hatched before 9th June, with a median hatch date of 17th May (population median this year, 16th May), giving the following sample sizes: for parent mass change, 106 adults in 58 nests; for survival measures, 189 eggs in 63 nests; and for chick growth, 134 chicks in 59 nests (table 3.2).

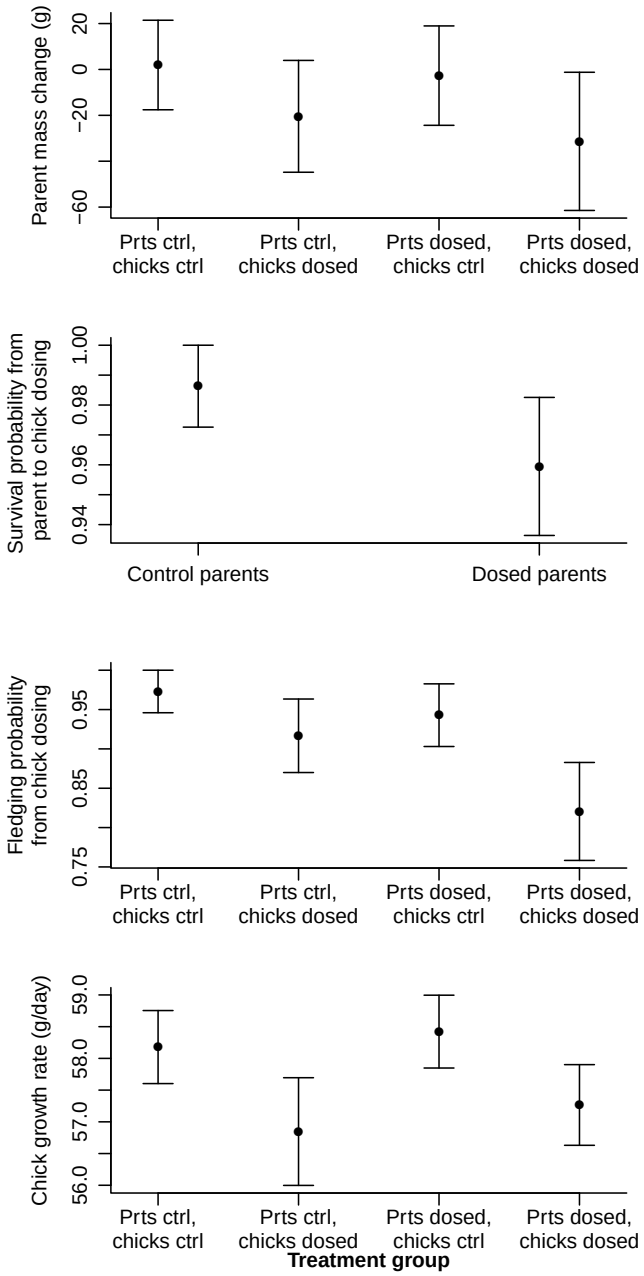


Figure 3.1: Means for each treatment group of all response variables tested: parent mass change during the experimental period, chick survival and chick growth rate. For space, drug-treated groups are shown as “iverm”. For illustration, chick survival is presented in two ways (c.f. three survival measures modelled): from parent treatment (before hatching) to chick treatment (age 10–12 days) for adult treatment groups, and from chick treatment to fledging for all treatment groups.

3.3 Results

Anti-nematode treatment of both parents and chicks had significant effects across the family (overview in fig. 3.1). However, parents were more strongly affected by chick treatment and chicks by parent treatment than either generation was by their own treatment.

3.3.1 Parent mass change

Anti-nematode treatment of parents did not affect their mass change through chick-rearing (table 3.3). However, parents' mass change was influenced by anti-nematode treatment of their chicks, with opposite effects in early and late breeders (table 3.3). In earlier nests, parents of treated chicks gained weight compared to controls, but in later nests, parents of treated chicks lost weight (fig. 3.2). The sexes did not differ in this relationship, nor did adult treatment change the way parents responded to chick treatment (table 3.3).

3.3.2 Chick growth rate

Anti-nematode treatment of parents did not affect their chicks' growth rate (table 3.4). There was a non-significant trend for chick treatment to reduce chick growth rate, with drug-treated chicks growing 1.2 g/day more slowly than control chicks (drug-treated chicks 57.1 ± 0.5 g/day, control chicks 58.3 ± 0.4 g/day; table 3.4). Treatment effects were similar throughout the season and for chicks of all ranks and sexes (table 3.4). Adult and chick treatment did not interact to affect growth rate (table 3.4).

3.3.3 Chick survival

Anti-nematode treatment of adults influenced their chicks' survival, increasing it in early season but decreasing it in late season (fig. 3.3). This difference in treatment effect between birds nesting before or after the median was significant (hatch date fitted as a two-level factor), though marginally non-significant when continuous hatch date was fitted. The model had the same explanatory power whether date was fitted as a continuous or categorical variable (table 3.5). The effect of adult treatment on chick survival did not differ between chicks of different sex or rank (table 3.5). Survival after chick treatment was not affected by chick or adult treatment, either as main effects,

Table 3.3: The fits of all models testing the effect of chick and adult treatment on parents' mass change across the experimental period, and a full description of the best fit model. ΔAICs are presented relative to the background model (sex * hatch date).

	Model ΔAIC	
Model	**Adult treatment**	**Chick treatment**
Main effect only	2.0	1.5
Interacting with:		
Hatch date	2.8	−0.9
Parent sex	0.8	3.3
Hatch date * sex	1.7	2.4
Adult treatment	–	1.7
Adult trt * hatch date	–	2.5

***Best fit model*:**

Parameter	**Estimate**	**Std. error**	**t-value**	**p-value**
Chick treatment * hatch date	−7.4	3.6	−2.05	0.046

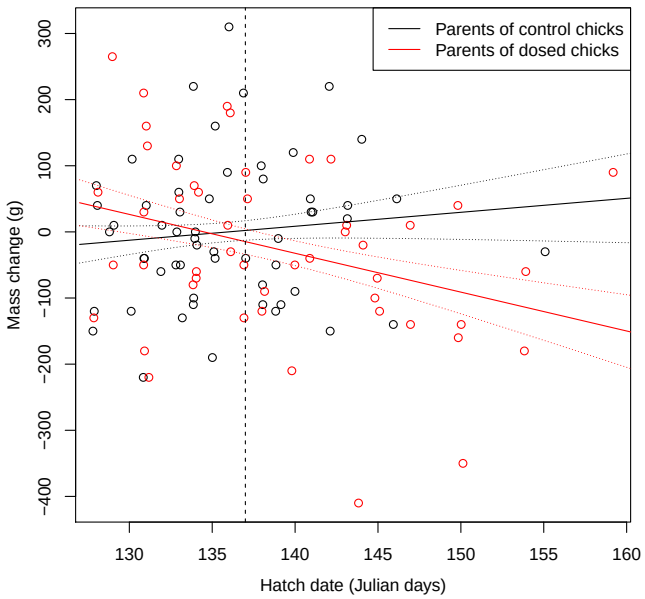


Figure 3.2: Parental mass change through the experimental period for parents of control (black dashed line, open symbols) and drug-treated (red solid lines, filled symbols) chicks, for birds nesting at different times in the season. Median hatch date was 137, i.e. 17th May. The plot shows the fitted relationship ± 1 standard error.

Table 3.4: The fits of all models testing the effect of chick and adult treatment on chick growth rate, and the full description of the best fit model. All models include a background model of sex and rank, and ΔAICs are presented relative to this model.

	Model ΔAIC	
Model	**Adult treatment**	**Chick treatment**
Main effect only	1.5	−1.4
Interacting with:		
Hatch date	2.7	1.1
Sex	3.4	0.1
Rank	4.4	2.0
Sex * hatch date	7.5	4.5
Rank * hatch date	0.4	6.0
Adult treatment	–	1.6
Adult trt * hatch date	–	4.7

***Best fit model*:**

Parameter	**Estimate**	**Std. error**	**t-value**	**p-value**
Chick treatment	−1.33	0.73	−1.83	0.073

interacting with each other or interacting with chick sex or rank (table 3.5). However, because post-treatment mortality was low (11 deaths, 134 survivors), these tests may have limited power.

3.4 Discussion

In this study, we have shown that the fitness consequences of anti-nematode teratment in a wild bird during the breeding season are distributed among the host's family. We used a two-by-two treatment design to experimentally address potential correlations between parents and offspring, and found that, for parents' mass change and chick survival, the direct effects of anti-nematode treatment on the treated generation were less marked than its indirect effects on the other generation. Both cross-generational impacts of treatment were apparently positive for early breeders but negative for late breeders. This is, to our knowledge, the first study of family-wide impacts of par-

Table 3.5: Fits of models testing the effect of adult and chick treatment on three measures of chick survival – from parent treatment to fledging, from hatching to fledging and from chick treatment to fledging – and a full description of the best fit model for each. Some effects could not be tested for certain survival measures, as chick sex and rank were only assigned at hatching. ΔAICs are presented relative to the background model for each survival measure (detailed in table 3.1). Hatch date is shown fitted as a two-level factor (before or after median), which gave stronger results and had the same explanatory power as continuous hatch date (all $\Delta\text{AIC} < 1.0$).

	Model ΔAIC		
Model	**From parent treatment**	**Post-hatching**	**From chick treatment**
Adult treatment	2	1.9	0.8
Treatment * hatch date	0.3	−1.5	−1.1
Treatment * sex	–	4.9	−1.9
Treatment * rank	–	3.2	−0.5
Treatment * sex * hatch date	–	3.3	–
Treatment * rank * hatch date	–	3.5	–
Chick treatment	–	–	1.3
Treatment * hatch date	–	–	4.3
Treatment * sex	–	–	0.2
Treatment * rank	–	–	-0.8
Chick * adult treatment	–	–	2.3

Best fit models:

Parameter	**Estimate**	**Std. error**	**z-value**	**p-value**
From parent treatment				
Adult treatment * hatch date	−2.15	0.87	−2.47	0.013
Post-hatching				
Adult treatment * hatch date	−4.33	2.01	−2.15	0.031
From chick treatment				
Adult treatment * sex	−2.73	1.90	−1.43	0.152

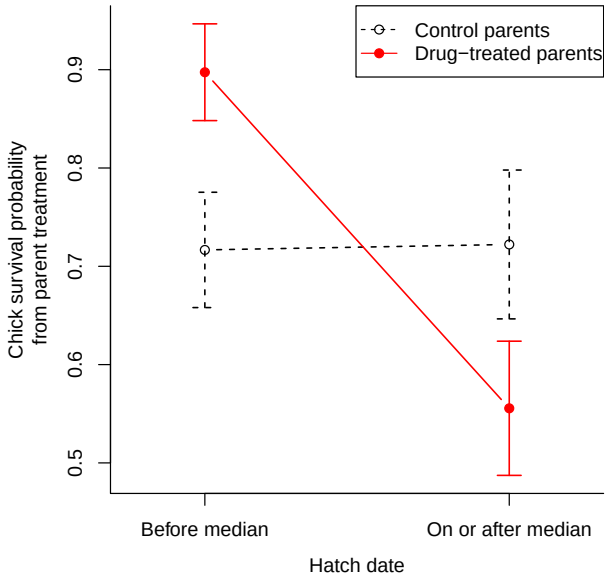


Figure 3.3: The effect of anti-nematode treatment of parents on the survival of their chicks, from the point of parent treatment (before hatching) to fledging, for birds breeding at different points in the season. Chicks of control parents are shown in black with open symbols and chicks of drug-treated parents in red with solid symbols. The plot shows mean values ± 1 standard error.

asitism in both parents and chicks, examining direct and indirect effects of parasite manipulation of both generations for all family members in a single experiment.

Several studies in a range of species have described cross-generational effects of parasitism in either direction, with parasite infection in parents reducing chick quantity and quality (e.g. Møller, 1994; Szép & Møller, 1999; Knowles et al., 2010a) or parasitism in chicks increasing parents' current reproductive effort or decreasing their future success (Christe et al., 1996; Richner & Tripet, 1999; Bize et al., 2004; Fitze et al., 2004b). However, we know of no previous study investigating the simultaneous impacts of parasitism in parents and chicks. Moreover, previous studies have concentrated on the effects of ectoparasites, which can move freely between family members. Thus, manipulating the parasite burden of certain individuals could also affect the burden of other family members, leading to an apparent cross-generational redistribution of parasite costs that is actually simply a direct effect of this correlated parasite burden. In several of the systems used to examine the impacts of parsaitism in families, such a correlation has been noted (e.g. Møller, 1994; Fitze et al., 2004b; Bize et al., 2004), and hence the relative importance of direct and indirect effects of parasitism for different family members remains unclear. In this study, we addressed this issue

by manipulating both parent and chick nematode burden. We found no additive effects when both generations recevied the drug treatment, suggesting that in our system, there is no strong correlation between worm burdens of parents and their chicks. In addition, treatment of endoparasites is more likely to affect treated individuals independently of other family members than ectoparasites; in our system, transmission of worms from parents to chicks is probably rare and from chicks to parents probably negligible (appendix A). Thus, the impacts of treatment we found across generations are likely not linked by shared parasite burdens, but by the effects of parasitism on each host and on its interaction with other family members. However, it should be noted that we do not have data to explicitly test nematode burdens in this experiment and cannot demonstrate the efficacy of the drug. Nonetheless, its efficacy has been demonstrated in chicks in two other years by using faecal egg counts and endoscopy (chapter 2, appendix A) and in parents in three other years using endoscopy and by its effect on chick survival (Burthe et al., 2013; Reed et al., 2008).

The effects of parent and chick treatment were strikingly similar in three ways. Firstly, both treatments had stronger indirect than direct impacts: parent treatment did not affect parents' mass change but altered their chicks' survival, and chick treatment only weakly reduced their growth rate but had a more substantial impact on their parents' mass change. Secondly, this held even when treatment effects appeared detrimental, with the natural intensity of infection of parents and chicks in late nests apparently benefitting their family members. Thirdly, both indirect effects depended on hatch date, with treatment having apparently positive consequences in early nests but apparently negative consequences in late nests. This is unexpected, as we would predict from the seasonal decline in breeding success that later breeders should suffer greater marginal costs of a parasite infection. Reed et al. (2008) found such an effect of anti-parasite treatment of adult shags on chick survival, but we found the opposite, both for adult and chick treatment. This may be linked to two aspects of how parasites affect their hosts that could vary through the season: extrinsic influences of increasing parasite prevalence, and a decline in the intrinsic overall quality of breeders, which could include immune function.

A recent study of *in situ* nematode burden of adult shags has demonstrated a seasonal increase in nematode prevalence in the same year as this study (Burthe et al., 2013), which predicts that anti-nematode treatment should be more beneficial later in the season. Our opposing results suggest a role for coinfection dynamics in our results: nematode infection has been shown in many species to influence the impact of other

parasitic infections (reviewed by Pedersen & Fenton, 2007; Ezenwa & Jolles, 2011; Tompkins et al., 2011), either by modulating host immune responses or by competition between parasites for host resources (Graham, 2008). Ivermectin treatment has recently been shown in wild mice to increase cestode and coccidian infections as well as reducing nematode burdens, presumably through a perturbation to the co-infection dynamics between these gastrointestinal parasites (Pedersen & Antonovics, 2013). If our late breeders hosted more nematodes, treatment may have caused a greater increase in the other parasite populations with negative effects for the bird. The co-infection dynamics of gastrointestinal parasite communities is a line of enquiry that could be fruitfully pursued in this system. Early- and late-nesting birds could also differ intrinsically, leading to changing treatment effects with hatch date. Later breeders tend to be younger or poorer quality (Daunt et al., 1999, 2006a), but we have shown that parental age and condition do not underlie our results. Instead, the seasonal change could be to the value of a current breeding attempt, altering the trade-off between current and future reproductive investment. A seasonal shift in investment decisions has been shown in two bird families: Alpine swifts (*Apus melba*, Apodiformes) and European starlings (*Sturnus vulgaris*, Passeriformes) nesting early in the season both preferentially fed chicks in poor condition, indicated by experimentally reduced plumage UV reflectance, whereas late nesters prioritized chicks in good condition (Bize et al., 2006). If shags apply similar rules, drug-treated (better-condition) chicks would receive less parental investment in early nests, allowing parents to gain mass themselves, whereas late in the season, treated chicks would receive more investment, making parents work harder and thus lose weight, as indeed we found for the effect of chick treatment on parents' mass change. Whatever the mechanism, it is notable that these between-individual differences in treatment effect may be of little consequence to the role of parasitism in shag population processes. Chicks hatched earlier in the season are more likely to recruit (Harris et al., 1994), so the way in which late breeders react to parasitism could be negligible at the population level if they contribute little to the breeding population.

The cross-generational link is likely to be closely associated with the availability of resources, i.e. energy and/or nutrients, to different family members. This is a currency that is shared and can be "traded" both between individuals and between physiological processes within an individual (Stearns, 1992). Investment of resources is also an important aspect of host defences against parasites, and in addition gastrointestinal nematodes may directly compete with their host for resources (Norris & Evans, 2000;

Colditz, 2008; Atkinson et al., 2009). Within a family, distribution of resources is the most commonly studied expression of evolutionary conflict between individuals (Godfray, 1995; Mock & Parker, 1997; Parker et al., 2002; Smiseth et al., 2008), and thus provides a well-established framework in which to consider the family-wide distribution of costs in the form of individuals witholding (parents) or demanding/taking (chicks) resources from others. We present a detailed analysis of how behaviour governing intra-familial resource distribution is affected by parasitism in chapter 4. Transfer of parasite costs between family members could also be mediated by cross-generational transfer of immunity. However, this can be excluded as an explanation of our results for two reasons: immunity can only be transferred between certain family members (maternal antibodies to chicks), and in birds this transfer only occurs before laying, so cannot account for the effects of our treatment, which began after laying.

From an ultimate perspective, the transfer of costs of parasitism to other family members could arise in two ways: either as a passive consequence of direct impacts on the host, or as an adaptive mechanism by which the host reduces the direct effects of infection on its immediate fitness. Commonly, cross-generational fitness consequences of parasitism are interpreted as a passive consequence of the direct effects of infection on the host (Christe et al., 1996; Bize et al., 2004; Knowles et al., 2010a), with parasitized individuals witholding resources from other family members as an unavoidable result of investment in their own parasite defences. On the other hand, transferring parasite costs could be an adaptive mechanism by which an individual might reduce costs to its immediate fitness and preserve its own reproductive value (Forbes, 1993). These explanations have different implications for the host's inclusive fitness, but are not mutually exclusive and thus difficult to tease apart. For example, parasitized parents may reduce provisioning, with a resulting decrease in chick survival (Knowles et al., 2010a). If this is a passive consequence, parents' current and future reproductive success may both be impaired, whereas if it is an adaptive response, reduced offspring survival may be offset by increasing parents' future reproductive potential. In our experiment, we found only weak (for chicks) or non-existent (adults) direct effects, suggesting an adaptive component to our findings: that hosts invest in their own defence against parasites, at a cost to their family members, to reduce or avoid costs to its individual fitness. The passive and adaptive hypotheses could be tested by examining how a host's future reproductive success is affected both in cases of apparent benefit and apparent detriment of treatment (early and late breeders, respectively), which we investigate in chapter 5. Whether passive or adaptive, we might expect relatively little

cross-generational transfer of parasite costs in a productive year like our study year (Newell et al., 2011), which suggests that our findings are robust to environmental variability despite the study only covering one breeding season.

Selection for individuals to avoid costs of parasitism to themselves could be expected to be stronger in long-lived, iteroparous species, where breeders stand to gain from witholding current effort in favour of future investment (Stearns, 1992). The shag breeds 10–15 times in its life, occasionally more, and produces up to four offspring per breeding attempt, so the value to parents of each chick may be relatively low and thus transferring costs of relatively little consequence to parents' inclusive fitness. Moreover, overwinter mortality of shags is vulnerable to extreme weather and thus stochastic (Frederiksen et al., 2008), so individuals may gain more from investing in their own condition and thus survival than in uncertain outcome of their family members. In chicks, too, we expect considerable selection for mechanisms to avoid costs during growth. The developmental environment of nestlings is crucial to their future success and could have life-long consequences for their morphology and behaviour (reviewed by Monaghan (2008), so the potential pay-off for a chick of maintaining optimal developmental conditions may be considerable. However, if parasite costs are traded in resources, physical control of these resources may constrain the outcome of the conflict over the distribution of costs (Royle et al., 2002). A family member who has greater control of resources may be more able to reduce the costs of infection to itself by monopolizing those resources and thus transferring costs to other individuals. In shags, parents may have the greater control: we have observed that a chick is never fed without begging, but begging does not guarantee a feed (chapter 4). Our finding of a weak direct impact of treatment in chicks, but no direct impact of treatment in parents, supports this idea.

In summary, anti-nematode treatment of both adult and juvenile shags had greater impacts for other family members that the treated host, suggesting that the costs of parasitism can be distributed between family members. This study indicates that the impact of parasitism during reproduction must be considered in a context of intra-familial conflict, as we have previously demonstrated within a generation (Reed et al., 2012; chapter 2), and provides a novel insight into how the costs of parasitism are distributed across the whole family. This could offer a fresh perspective on the currency for parent–offspring conflict. Notably, our strong indirect effects of anti-nematode treatment indicate that measuring the impacts of infection only on the host could underestimate the overall impact of parasitism at the population level. Our findings reflect

the complexity of both intra-familial conflict and an individual's strategy to cope with the costs of parsitism, and illustrate the important role that parasite infection plays in host ecology and life-history.

CHAPTER 4

Anti-parasite treatment influences conflict-related behaviour in parents and offspring

4.1 Introduction

The distribution of resources among members of a family is crucial to the outcome of a reproductive attempt (Stearns, 1992; Mock & Parker, 1997; Parker et al., 2002). In sexually reproducing species, all family members are expected to be in evolutionary conflict over how resources such as food are partitioned among them, with each demanding more resources than is optimal for others to allow them to have (Trivers, 1974). Such conflicts of interest have been well described between siblings, between parents, and between parents and offspring (Mock & Parker, 1997; McNamara et al., 1999; Parker et al., 2002; Royle et al., 2012).

In altricial species, this evolutionary conflict is often manifested in three main aspects of behaviour governing the distribution of resources: offspring signalling their demand for food to parents, parents provisioning offspring in response to those signals, and offspring competing with each other for access to parental provisioning. Offspring in many species express demand to their parents in order to solicit a feed. This

commonly takes the form of stereotyped begging displays that are honest signals of short-term or long-term offspring need, but in some species may also reflect offspring quality and be influenced by interactions between siblings (Kilner & Johnstone, 1997; Royle et al., 2002; Grodzinski & Lotem, 2007; Mock et al., 2011). Secondly, parents may be selective about how they respond to these signals, i.e. which offspring they feed (Parker et al., 2002; Lessells, 2002; Kilner, 2002). Thirdly, siblings may compete directly with each other. Outright aggression between siblings is common and is particularly well-studied in birds (reviewed in Drummond, 2001; in mammals, Hudson & Trillmich, 2007). Often, larger dominant siblings gain preferential access to parental provisioning to the detriment of subordinate nest-mates (Drummond, 2006).

Thus, offspring signalling, parental provisioning and sibling competition are essential in shaping the social environment of the family, how resources are allocated and hence the success of a breeding attempt. These behavioural interactions can also be altered by other influences affecting family members' resource demands that are external to the social environment. One important such influence is parasite infection. Parasitism is costly for the host, redirecting resources from other physiological processes to immunity and directly depriving the host of resources (Colditz, 2008; Atkinson et al., 2009). Parasitism has been shown to be detrimental to all family members, reducing offspring survival (Fitze et al., 2004b) or condition (O'Brien & Dawson, 2008) as well as parents' condition (Stien et al., 2002) and future breeding success (Bize et al., 2004). Moreover, the costs of parasitism may not be restricted to the host, so infection in one individual may also have fitness consequences for other family members: parasitism of chicks may affect parental condition and future reproductive potential, and vice versa (e.g. Christe et al., 1996; Bize et al., 2004; Knowles et al., 2010a; chapter 3, this thesis). This suggests that the behavioural dynamics governing resource distribution among family members could be an important link between parasite infection and its impact on breeding success.

Several studies have demonstrated the potential of parasitism in both offspring and parents to affect all three aspects of intra-familial behaviour. Ectoparasitism of chicks increases begging rate but decreases growth rate in great tits *Parus major*, indicating that parasitism increases chicks' need for resources and hence influences signalling (Christe et al., 1996). Similarly, investment in a stimulated immune response increases behavioural begging intensity in female barn swallow *Hirundo rustica* chicks (Romano et al., 2011). Parental provisioning rate may increase to meet these extra resource demands, as has been shown in broods of great tits infected with fleas and blue

tits *Cyanistes caeruleus* infected with blowflies (Christe et al., 1996; Hurtrez-Boussès et al., 1998). The outcome of sibling competition may also be altered by offspring parasitism: in great tits, parental food allocation within a brood was more equal in experimentally deparasitized broods than in broods with natural levels of flea infection (Christe et al., 1996). However, to our knowledge no study has specifically examined parasite-induced changes to the underlying competitive behaviour. In addition, the impact of parasites may be asymmetrical among siblings that differ in age and size, as we expect larger siblings to suffer smaller marginal costs of a given parasite burden and to be better able to access parental resources to counter those costs. These size differences may be compounded or counteracted by variation through the clutch in pre-hatching maternal investment of antibodies, and hence defence against parasites, as well as post-hatching parental provisioning, and hence chick condition (Mock & Forbes, 1995; Hargitai et al., 2006). Indeed, several studies have suggested that parasite-induced behavioural change may underlie observed differences between family members in susceptibility to the detrimental effects of parasitism (O'Brien & Dawson, 2009; Knowles et al., 2010a; Reed et al., 2012).

Parents' own parasite infection can also change their behavioural interactions with their offspring. Treating blue tit mothers against blood parasites increases their provisioning rate, presumably because they can invest resources in their chicks that are otherwise required to deal with an infection (Tomas et al., 2007; Knowles et al., 2010a). As with offspring, parents may differ both in how they respond to the costs of parasitism and in their behaviour. In European shags *Phalacrocorax aristotelis*, anti-parasite treatment of parents increased mothers' foraging effort during chick-rearing but not fathers' (Reed et al., 2008). Different parents may also have different provisioning rules. In Alpine swifts *Apus melba*, for example, parents nesting early in the season preferentially feed chicks with low plumage UV reflectance (indicating poor chick condition), while late nesters favour chicks with high UV reflectance (Bize et al., 2006), and in canaries *Serinus canaria* and Manx shearwaters *Puffinus puffinus*, mothers are more sensitive to chick signalling than fathers (Kilner, 2002; Quillfeldt et al., 2004). Thus, mothers and fathers may differ in their responses to chick parasitism.

The studies to date illustrate two important considerations of the role of parasites in resource distribution within a family. Firstly, both parent and offspring behaviour may be directly impacted by parasitism, and both are important to resource distribution and thus breeding success. Secondly, intra-familial interactions can be influenced by parasite infection in both parents and offspring, as parasitism of parents may indi-

rectly affect offspring and vice versa. The outcome of intrinsic asymmetries between chicks in parasite impacts could thus vary depending on the influence of parasitism on the behaviour of other family members, as a chick's social environment has been shown to strongly influence its success (Forbes, 2011). Therefore, the parasite burdens and behaviour of all individuals in a family must be considered in order to gain a full appreciation of the role of parasitism for resource distribution. Despite this, few studies examine both the direct and indirect effects of parasitism on all family members' behaviour (but see Christe et al., 1996; Moreno-Rueda & Redondo, 2012), and to our knowledge no previous study has investigated how simultaneous infection in both parents and chicks contributes to resource distribution between family members within a single experimental framework. Furthermore, chick and parent parasite burdens may not be independent: we expect family members to share environments and hence parasite exposure as well as genetic bases for immunity. In addition, parents may act as a source of parasites to their chicks or vice versa (see introduction to chapter 3, section 3.1). In order to isolate the effect of either parent or offspring parasitism, therefore, the other must be experimentally controlled. In addition, parasite burdens and their impacts are likely to vary between the years in which separate components of intra-familial interactions have been investigated to date, so the relative roles of parent and offspring parasitism on behaviour in a given reproductive attempt are not clear.

In this study, we investigate how behavioural interactions in families of European shags *P. aristotelis* are influenced by gastrointestinal nematode infection of both parents and chicks. We have previously shown in shags that anti-nematode treatment of parents influences chick survival and that treatment of chicks influences parents' mass change through the breeding period (Reed et al., 2008; chapter 3), indicating that behavioural interactions between parents and chicks may underpin the family-wide effects of parasitism. Treatment of parents has been shown to increase mothers' foraging effort during chick-rearing (Reed et al., 2008), which suggests that parasite infection in parents could limit the amount of food that they can distribute to chicks. Further, parasite infection constrains the growth of last-hatched chicks more than that of older siblings, but only in less productive years, suggesting that parasite effects on chicks may depend on sibling competition over limited resources (chapter 2). Behavioural dynamics between all family members are thus likely to be important in mediating the physiological impacts of parasitism. Moreover, in shags, chick begging signals, parental provisioning and sibling competition in the form of aggression can all be readily observed but have not previously been described in detail. Intra-familial

conflict behaviours in this seabird can provide an informative contrast to the shorter-lived, larger-brooded passerines more commonly used as model systems in studies of resource distribution within a family.

We use anti-nematode treatment of chicks and/or parents in a fully factorial design to investigate the effects of parasitism across the family for these key intra-familial interactions across two breeding seasons. We predict that provisioning will reflect chick begging, and that chick treatment will affect chicks' ability or need to beg, and hence the amount of food they receive. We expect a greater effect of treatment on last-hatched chicks' behaviour than on their older siblings, mirroring the effect of treatment on chick growth rate (chapter 2), and that these within-brood differences in behaviour will be stronger in our first experimental year, which showed greater within-brood differences in the impact of anti-nematode treatment on chick growth rate than the second year (chapter 2). We predict that drug-treated parents will have more surplus resources and therefore provision offspring more, but do not expect treatment of parents to affect sibling aggression. Instead, we expect that chicks suffering greater marginal costs of parasitism will gain a greater competitive benefit from treatment, such that siblings in drug-treated broods will be more evenly matched in aggression.

4.2 Methods

This study exmines the effects of endoparasitism on behavioural interactions governing resource distribution within the family in European shags. We used video data from two years of anti-nematode treatment experiments to examine how chick begging, parental provisioning and sibling competition in the form of aggression change with treatment. All procedures were carried out under UK Home Office licence (licence nos. PIL 60/12450, PPL 60/3444), all ringing under licence from the British Trust for Ornithology, and experiments under a National Nature Reserve research licence from Scottish Natural Heritage (licence nos. MON/RP/115 & MON/RP/124).

4.2.1 Study species

This study was conducted on the breeding population of shags on the Isle of May, south-east Scotland (56° 11 N, 2° 33 W) in 2010 and 2011. The two years differed markedly in their productivity (2.04 chicks fledged per nest in 2010, 1.52 in 2011, c.f. mean of 1.41 across last decade; Newell et al. (2011)) and in the impact of chick

treatment on chick growth (chapter 2). Shags have a modal clutch size of three (Snow, 1960), where the third chick hatches c.2 days after its older siblings, is often smaller for a substantial part of its development and has lower survival than its older siblings (Snow, 1960; Stokland & Amundsen, 1988; chapter 2). Adult and nestling shags are infected with gastrointestinal nematodes through their fish diet (Anderson, 2000; Fagerholm & Overstreet, 2008), predominantly *Contracaecum rudolphii* and larval *Anisakis simplex* (Reed et al., 2008; Burthe et al., 2013). These nematodes feed on ingested fish, competing with the host for resources, and attach to the bird's stomach wall, causing tissue damage (Anderson, 2000; Fagerholm & Overstreet, 2008; Moravec, 2009). Nematode infection also causes the host to invsest resources in immunity (Colditz, 2008), and indeed, anti-nematode treatment has shown that infection is costly for both adult and nestling shags (Reed et al., 2008, 2012; chapters 2 and 3).

4.2.2 Experimental methods

In 2010, we examined the effect of endoparasitism on chicks, treating whole broods with an anti-nematode drug and comparing these to sham-treated control broods (detailed protocol in chapter 2). In 2011, we examined the family-wide effects of anti-nematode treatment in all family members including parents as well as their offspring. We treated parents and/or chicks and compared them to controls in a two-by-two factorial design (detailed protocol in chapter 3). This gave two groups that repeated the 2010 design (only chicks drug-treated and neither chicks nor parents drug-treated) and an additional two groups in 2011 only to investigate the impact of infection in parents (only parents drug-treated and both chicks and parents drug-treated).

In both years, we selected nests of 3 eggs (the modal clutch size) for experimental treatments. In 2010, parents were not manipulated, but in 2011, we caught both parents on all experimental nests at 3–7 days before predicted hatching (based on observed lay date and a 35-day incubation period; Potts et al. (1980)) and treated them with either a broad-spectrum anti-nematode drug (ivermectin, Panomec©by Merial, 1% wt/vol) or a saline control at a dose of 0.7mg/kg (0.1–0.15ml), with treatment group randomly assigned to nests matched for lay date and colony area. In both years, hatching was determined accurately to within 2 days by regular nest visits. Newly hatched chicks were marked individually, assigned ranks according to hatching order (A, B and C chicks the first-, middle- and last-hatched respectively) and blood sampled for molecular sexing (Griffiths et al., 1996). In both years, when the A chick was 10–12 days old, all chicks

in a brood were treated with 0.05ml of either ivermectin or a saline control. Chick treatment was randomized across nests but matched according to hatch date, colony area, and in 2011, parent treatment. When the A chick was aged 22 days, we collected behavioural data using video observations of interactions within the nest.

4.2.3 Filming protocol

We focused our filming on nests with three chicks. Previous work has shown that the last-hatched chick in a brood of three responds differently to anti-nematode treatment than its two older siblings, whose responses are similar (Reed et al., 2012; chapter 2). Moreover, the competitive dynamics in a brood of three may differ qualitatively from smaller broods, making direct comparison of different brood sizes difficult.

We used a handheld digital video camera (Toshiba®Camileo P30) attached to a weighted tripod placed at least 2m from the nest to reduce disturbance. A waterproof covering for the camera allowed continuous filming regardless of weather. Before filming, chicks were removed from the nest and marked individually with water-based correction fluid, using a randomized number of dots on the head and bill of each chick. We observed no immediate ill effects of marking and all filmed chicks fledged successfully. As soon as the chicks were returned to the nest, the camera was started and left to record until its power ran out. In 2011, we weighed chicks during marking and again within 30 minutes of filming finishing to measure mass change during the video for a subset of nests. This was as a proxy for how much food the chick had obtained, although we did not account for excretion and digestive efficiency which also contribute to this measure (Grodzinski & Lotem, 2007).

In 2010, we filmed nests over a range of chick ages, throughout the day and for varying lengths of time (chick age 12–38 days, video start time 05:00–20:00, video length 0.4–11 hrs; and some nests filmed more than once) to examine the impact of these factors on behaviour in the nest and to refine our filming protocol for 2011. We wished to test the effect of chick age on behaviour as in the related blue-footed booby *Sula nebouxii*, sibling conflict behaviour becomes less pronounced through development (Valderrábano-Ibarra et al., 2007). Chick age did not bias detection of any behaviours in 2010 (all $p > 0.1$), so in 2011 we chose to film when the oldest chick was aged 22 days, which fell in the first few days when all chicks in a brood would be large enough to be reliably observed. Video length did not affect the detection of begging or feeding (duration per hour by video length, both $p > 0.6$), but aggression was

overrepresented in short videos (effect size -0.58 ± 0.20, $p = 0.007$). This effect was driven by the shortest videos and excluding 2 videos less than 1 hour long removed the relationship ($p = 0.258$). Therefore, in 2011 we took videos 2–3.5 hours long (camera battery life, which varied with conditions). Time of day had no effect on the detection of any behaviours (all $p > 0.1$, for both linear fits to test for a steady change through the day and quadratic fits to test for peaks in the morning and evening) and in 2011 we filmed throughout the day.

4.2.4 Behaviour in the nest

Shag chicks are altricial, confined to the nest and fed entirely by their parents until aged c.50 days (Snow, 1960). Chicks have a sterotyped begging display to which parents respond by providing food, but the relationships between begging and provisioning have not previously been described in detail in this species.

We recorded the three main aspects of intra-familial interactions – chick begging, parent provisioning and sibling competition as aggression – for the entire duration of each video. All behaviours were assigned to individual family members, all of known sex and, for chicks, hatching order.

4.2.4.1 Chick begging

The begging display consists predominantly of a slow, horizontal weaving of the head, often accompanied by a series of long, shrill squeals. This is punctuated with short bouts of rapid shaking of the bill from side to side, usually directed towards the parent's bill and often associated with a more upright posture, which we refer to as "rapid" begging. Begging occurs as a continuous bout interspersed with long periods of inactivity or sleeping. We focussed on the begging display as a component of chick signalling that could be characterised and quantified, but note that the amount of begging is likely to be only a part of a chick's signal to parents. The signal may not only include other components, such as vocal signals, which we were not able to measure, but intensity of the signal may also be reflected more in these other components than in the amount or quality of the begging display.

To quantify levels of begging, we recorded the total length of each begging bout and the duration of rapid begging within it. This gave us total begging time and total rapid begging time for each video.

4.2.4.2 Parental provisioning

During a feed, the parent opens its bill to allow the chick to insert its head into the parent's crop, where it feeds directly on regurgitated fish.

To quantify provisioning, we noted whether each begging bout resulted in a feed and the duration of each feed, which we defined as the period for which the base of the chick's bill was inserted beyond the base of the parent's bill. This gave a total duration of feeding for each video.

4.2.4.3 Sibling aggression

Shag chicks exhibit overt aggression towards each other. At the age when chicks are large enough to be reliably observed, this is visible as the aggressor biting the victim several times in rapid succession.

To quantify aggression, we recorded the duration of each aggressive bout and the number of bites made by the aggressor during it. This gave a total duration and a total number of bites for each video.

4.2.5 Statistical analysis

We analysed all behaviours in terms of duration, which was well correlated with other measures examined: total begging duration was closely associated with the number of bouts (mixed model $p < 0.001$; in linear model without nest as random, $r^2 = 0.43$), total feed duration with the number of feeds (mixed model, $p < 0.001$; linear model, $r^2 = 0.91$), and total aggression duration with the total number of bites (mixed model, $p < 0.001$; linear model, $r^2 = 0.96$) and the number of aggressive bouts (mixed model, $p < 0.001$; linear model, $r^2 = 0.83$).

We standardized all measures as a rate (duration per hour) in order to account for differences in video length. Videos under 1 hour long were excluded from all analyses, and for videos over 1 hour across both years, video length did not influence rates or the effect of treatment (as single main effect, in addition to or interacting with treatment in all models, $p > 0.1$). All analyses examined rates of behaviour at the level of individual chicks. At the level of the nest, no behaviours were significantly affected by treatment (treatment effects on total begging, provisioning and aggression across all brood members, $p > 0.1$).

We modelled the rate of begging, provisioning and aggression as response variables in relation to treatment. In addition, we used begging as an explanatory variable for

provisioning and aggression as an explanatory variable for provisioning and begging. In 2011, we also examined the effect of treatment and all three behavioural variables on chick mass change. All variables were modelled across both years with year fitted as a fixed factor (with only two levels, fitting year as a random factor would not be appropriate). We tested chick treatment as a main effect and interacting with year, chick rank and chick sex. This examined whether the effect of treatment on behaviour varied between years and whether siblings differed in their behavioural responses to treatment, as both factors have been previously demonstrated to shape chicks' physiological responses to treatment (Reed et al., 2012; chapter 2). Because our sample in 2010 was small compared to 2011, treatment differences between years might not give a statistically significant interaction. Therefore, we qualitatively compared years by testing each year separately for treatment effects. In 2011 only, we also tested for the effect of adult treatment as a main effect, interacting with chick sex or rank, and additionally interacting with chick treatment. In this year, the physiological impact of both chick and parent treatments varied with hatch date (chapter 3), but three-chick nests, the focus for our behavioural questions, were predominantly early in the season (only 5 of 22 nests on or after the median hatch date), when parent treatment increased chick survival and chick treatment improved parents' condition. No behaviours or treatment impacts on behaviour differed across this range of hatch dates, so hatch date is not included in any models presented below.

All analysis was done in R 2.15.1 (R Development Core Team, 2011). Where necessary, we transformed response variables to normalize model residuals (table 4.1) so that parametric mixed modelling could be used, allowing nest to be fitted as a random effect to account for the non-independence of siblings in a brood. All analysis used linear mixed effects models in the R package nlme (Pinheiro et al., 2012), apart from the analysis of separate ranks, which used simple linear models in the package stats (R Development Core Team, 2011). We conducted model selection primarily based on AICs, and also considered p-values in the case of equivalent model fits ($\Delta\text{AIC} \leq -2.0$). All parameter estimates are given as the mean $\pm$ 1 standard error.

Overall, this analysis covers data from 21 chicks in 7 nests in 2010, of which 4 nests were filmed more than once, and 66 chicks in 22 nests in 2011 (table 4.2). When modelling both years simultaneously, we ensured comparability by restricting the dataset from 2010 to include only one video per nest (the video that was closest in age to 22 days for nests that were filmed more than once), and from 2011 to include only nests with control parents. Mass change data in 2011 was obtained for 54 chicks in 18 nests.

Table 4.1: Transformations applied to normalize the residuals of all behavioural rates.

	Transform		
Behaviour	2010	2011	Years combined
Begging	$\log(x+1)$	$\log(x+1)$	$\log(x+1)$
Rapid begging	$\log(x+1)$	$\sqrt{x}$	$\log(x+1)$
Provisioning	$\log(x+1)$	none	none
Aggression	$\log(x+1)$	$\sqrt{x}$	none

Table 4.2: Sample sizes as numbers of nests used in each year of the study. In 2010, 2 control and 2 drug-treated nests were filmed more than once; all these videos were included in analyses of 2010 only. For the overall analysis of the years combined, we restricted the videos we used to ensure comparability (details in text).

Year	**Control chicks**	**Drug-treated chicks**
Both years combined	10 nests	7 nests
2010	4 nests	3 nests
2011, control parents	6 nests	4 nests
2011, drug-treated parents	6 nests	6 nests

4.3 Results

4.3.1 Description of behaviours

4.3.1.1 Chick begging

Across both years, begging bouts lasted on average 62 ± 3.9 seconds across both years (range 2–420 seconds), and the inactive periods in between begging bouts frequently lasted over 30 minutes. Initiation of begging was unpredictable, sometimes occurring immediately after a chick woke up and at other times when it had been active for half an hour or more. Begging initiation was not closely tied to the arrival of a parent: of a total of 27 returns filmed across both years, only 3 immediately triggered a begging event (within 30 seconds), and another 7 were followed within 5 minutes by a begging bout. Begging appeared as likely to be initiated by other movement, such as the parent preening the chicks or siblings begging.

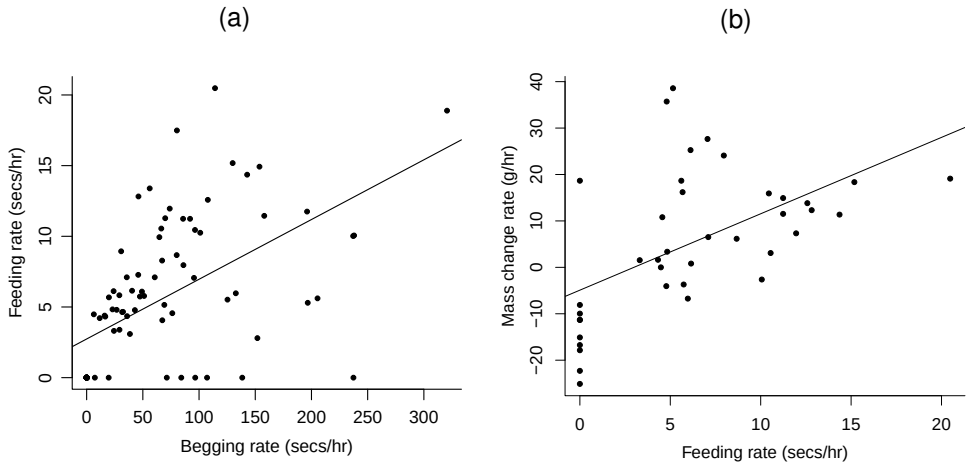


Figure 4.1: The fitted relationship between between begging rate and feeding rate across both years (fig. 4.1a), and the relationship between feeding rate and mass gain, investigated in 2011 only (fig. 4.1b). Across both years, an increase in begging of 60 secs/hr increased feeding by 1.8 secs/hr ($p < 0.001$, $r^2 = 0.31$). In 2011, a feeding rate increase of 1 sec/hr resulted in 1.6g/hr greater mass gain ($p < 0.001$, $r^2 = 0.28$).

It was rare for two chicks to beg at the same time; we only observed two siblings begging simultaneously in 22 out of 375 begging bouts across the two years (5.8%). In all these cases, the second chick initiated begging when its sibling was already begging and appeared (apart from in one nest) to attempt to obtain the feed solicited by the already-begging sibling. We only observed a second-begging chick take a feed on one occasion.

Begging was necessary to obtain food, as chicks were never fed without begging first. However, parents only responded to begging with a feed in $43 \pm 3\%$ of begging bouts.

4.3.1.2 Parental provisioning

A feed lasted on average 14.1 ± 0.4 seconds across both years. Duration of provisioning was best predicted by total begging time, and the relationship was not driven by chicks that did not beg and thus were not fed (table 4.3, fig. 4.1a). Mothers and fathers did not differ in their responsiveness to chick begging (for each begging bout: effect of parent sex on likelihood of obtaining a feed or duration of feed, all $\Delta\text{AIC} > 1.8$ from year-only model). In 2011, chicks were fed more for a given amount of begging than in 2010 (table 4.4).

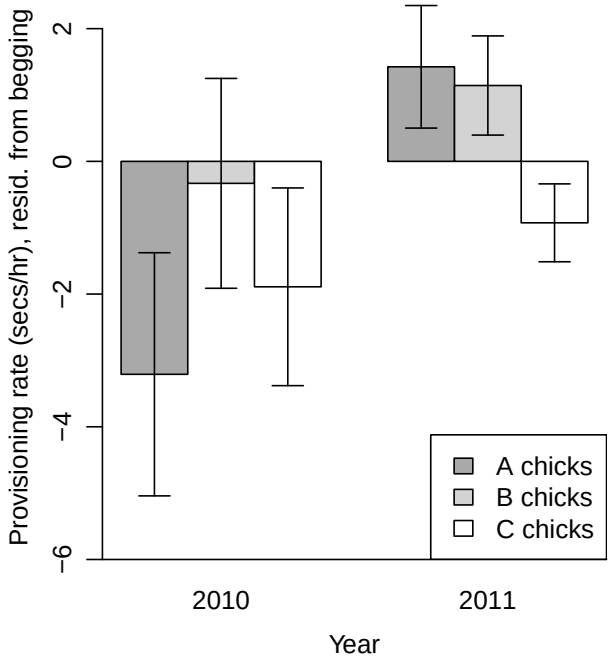


Figure 4.2: The difference in feeding rate, accounting for begging rate, between chicks of different ranks in 2010 (left panel) and 2011 (right panel). A chicks are shown in dark grey, B chicks in light grey and C chicks in white. Bars show mean values ± 1 standard error.

Table 4.3: Model fits (presented as ΔAICs from a year-only model) of relationships between begging and feeding rates, for all chicks and for the subset that excludes chicks that did not beg and thus were not fed. All models use data from both years, including data from both control and drug-treated adults in 2011, and contained a main effect of year.

	Feed duration per hour	
Explanatory variable	All chicks	Only begging chicks
Year only	0.0	0.0
Total begging time	−21.6	−7.3
Rapid begging time	−5.6	0.0
Proportion of begging rapid	−9.8	1.8

Last-hatched chicks were fed less than their older two siblings in 2011, but this pattern was weaker across both years (table 4.4; fig. 4.2). The effect was not driven by size differences between chicks (measured in 2011 only; feeding rate to C chick in relation to its mass difference from average A & B mass, absolutely or proportional, or in relation to A & B's total mass, all $p > 0.6$; all models include begging). In contrast to a previous study (Reed et al., 2008), we did not find that more male-biased broods were fed more frequently (per nest, feed duration predicted by number of sons, $\Delta\text{AIC} = 0.6$ from begging duration only), although productivity varied substantially across the years in that study and this work, (chapters 2 and 6).

In 2011, chicks gained on average 5.4 ± 2.5 g/hr of mass. Mass change was strongly associated with the total duration of feeding (table 4.4, fig. 4.1b) but was not affected by begging (in addition to feeding, begging or rapid begging $\Delta\text{AIC} > 0.9$ from feeding-only model). Chicks gained on average 1.7g per second of a feed, and the average feed size (among chicks that were fed, total number of feeds ÷ total mass change) was 24.5 ± 6.2g. The average feeding rate in this subset of chicks was 0.50 ± 0.05 feeds/hr, which during $\sim$17.5 hours of daylight (shags are not known to provision during darkness; K. Herborn, pers. comm.) suggests an average daily intake of 213g of food. An average growth rate of 58.7 g/day (chapter 3) implies that shag chicks assimilate 27% of their food intake into growth.

4.3.1.3 Sibling aggression

Aggression was commonly directed at the victim's bill, but could be directed at any part of the body. We never observed injuries resulting from aggression, either in video footage or while handling the chicks. As with begging, aggression occurred in concentrated bouts with long periods of inactivity in between and, similarly, there was no predictable pattern to the initiation of aggression.

Aggression rate was not correlated with begging or provisioning rates (for both years and in each year, all $\Delta\text{AIC} > 0.2$ compared to year-only and intercept-only models respectively).

4.3.2 Treatment effects

Anti-nematode treatment of nestling shags affected all aspects of behaviour in the nest: begging, provisioning and sibling aggression. Treatment of parents, on the other hand, did not affect any behaviour.

Table 4.4: Prameter estimates from models that explained associations between begging and provisioning variables ($\Delta\text{AIC} \leq -2.0$ from intercept-only model). Effect sizes are measured in g/sec of feed for mass change and in secs/hr for feeding rate. ΔAICs are shown compared to an intercept-only model for mass change and a begging-rate-only model for feeding rate. Results of other tests are given in the text.

Explanatory variable	ΔAIC	p-value	Effect size	t-value
Mass change				
Feeding rate	-11.5	< 0.001	1.65 ± 0.42	3.93
Feeding rate (all models include begging)				
Year	-3.6	0.026	2.9 ± 1.2	2.47
Rank (B chicks)	-3.3	0.615	0.5 ± 1.1	0.51
Rank (C chicks)		0.18	-1.4 ± 1.1	-1.36
2011: Rank (B chicks)	-5.9	0.931	0.1 ± 1.1	0.09
2011: Rank (C chicks)		0.039	-2.4 ± 1.1	-2.13

4.3.2.1 Chick signalling

Drug-treated chicks tended to beg more overall than control chicks, though not significantly so, irrespective of rank or sex (table 4.5; rank and sex main effects or interacting with treatment, $\Delta\text{AIC} > 0.1$ from year-only model). This was driven by the first year of filming: in 2010, drug-treated chicks begged for 96 seconds longer per hour than control chicks ($\Delta\text{AIC} = -4.6$ from intercept-only model, fig. 4.3). In contrast, in the second year, neither chick treatment nor adult treatment affected begging time, with chicks of all ranks and sexes responding similarly (all treatment main effects and interactions $\Delta\text{AIC} > 1.0$ from intercept-only model). Chick treatment had little effect on rapid begging with no significant effects overall or in either year (main effect and interactions with sex and rank: all $\Delta\text{AIC} > -1.3$).

4.3.2.2 Parental provisioning

Overall, chick treatment did not significantly affect parental provisioning rate, nor did this differ between chicks of different sex or rank (table 4.5; rank and sex main effects or interacting with treatment, $\Delta\text{AIC} > 1.9$ from year-only model). Nonetheless, in 2010, drug-treated chicks were fed for 5.8 seconds longer per hour than control chicks

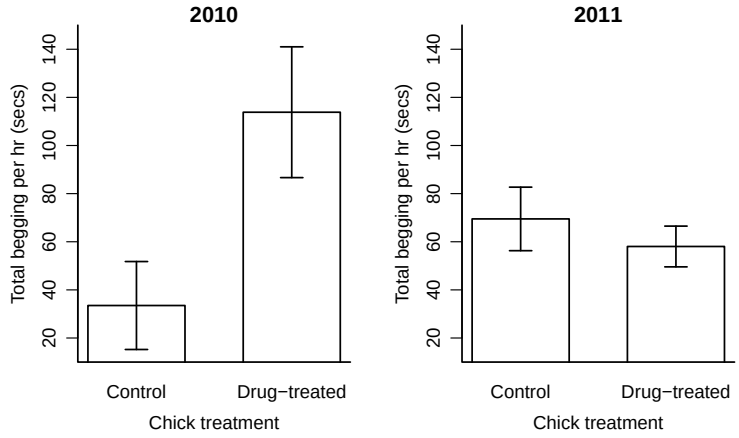


Figure 4.3: The effect of chick treatment on begging rate in 2010 (left panel) and 2011 (right panel). Bars show mean values ± 1 standard error.

(table 4.5; fig. 4.4). Chick treatment also increased feeding in 2011, although only for sons (table 4.5; fig. 4.4; sex and rank main effects $\Delta\text{AIC} > 1.1$ from intercept-only). There was no difference between chick ranks (rank * treatment interaction model, $\Delta\text{AIC} = 5.1$). Adult treatment did not affect feeding duration (main effect and interactions, including with chick treatment, all $\Delta\text{AIC} > 0.8$). The relationship between begging and provisioning was not influenced but either chick or adult treatment (time fed predicted by total begging, within each year and across both years, interaction of treatment with begging effort all $\Delta\text{AIC} > 0.8$).

Mass change (2011 only) did not vary with either chick or adult treatment as main effects nor treatments interacting with each other, with feed duration or with chick sex or rank (in addition to feed duration (best predictor of mass change), all $p > 0.1$, all $\Delta\text{AIC} > 0.2$ from intercept-only model).

4.3.2.3 Sibling aggression

Drug-treated chicks were aggressive for 1.6 seconds longer per hour overall than control chicks, with A chicks less strongly affected than their junior siblings (table 4.5; fig. 4.5). This was driven by our second year of filming, although both years showed qualitative similarities in the impact of treatments on the three ranks (fig. 4.5). Chick treatment did not significantly affect aggression in 2010 (main treatment effect and interactions, $p > 0.07$, $\Delta\text{AIC} > 0.4$ from intercept-only). In 2011, on the other hand, A chicks in drug-treated broods were less aggressive than their younger siblings (table 4.5). Adult treatment had no impact on sibling aggression (main effect and interactions, all $\Delta\text{AIC} = 0.8$ from intercept-only).

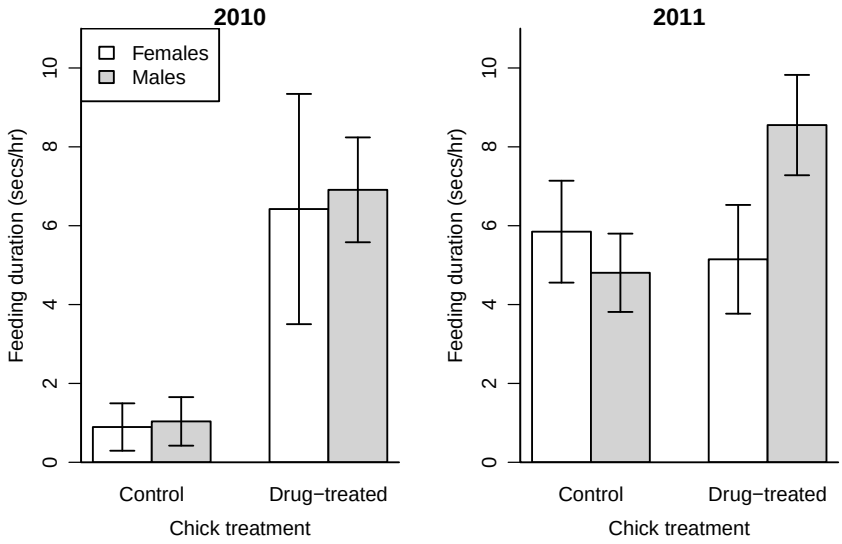


Figure 4.4: The effect of chick treatment on parental feeding rates to daughters (open bars) and sons (shaded bars) in 2010 (left panel) and 2011 (right panel). Bars show mean values $\pm$1 standard error.

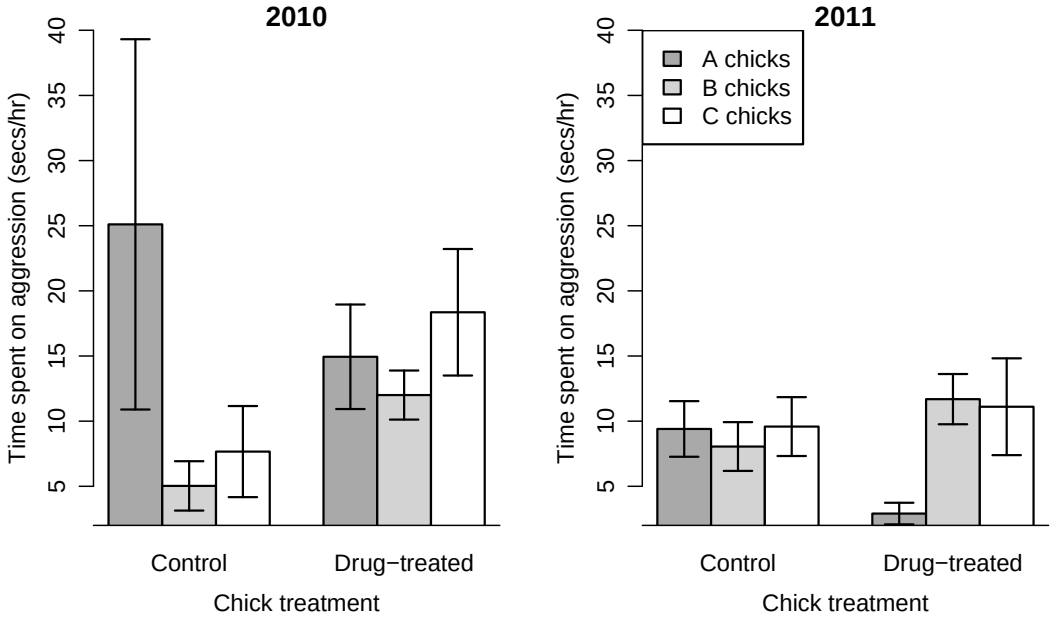


Figure 4.5: The effect of chick treatment on sibling aggression in 2010 (left panel) and 2011 (right panel) for chicks of different ranks. A chicks are shown in dark grey, B chicks in light grey and C chicks in white.

Table 4.5: Fits and parameter estimated of models examining the effects of treatment (abbreviated to trt. for space) on behaviour in the nest. Where no year is specified, the model fits both years of data. All effect sizes are in secs/hr. ΔAICs are given relative to a year-only model for the two-year datasets and relative to an intercept-only model for single-year datasets. Results of other tests are given in the text; adult treatment had no significant effects on any behaviour.

Response	**Explanatory variable**	**ΔAIC**	**p-value**	**Effect size**	**t-value**
Begging rate					
	Chick treatment (+ year)	-2.0	0.062	1.2 ± 0.6	2.03
Feeding rate					
	Chick treatment (+ year)	0.0	0.179	2.0 ± 1.4	1.41
	2010: Chick trt.	-5.7	0.026	1.2 ± 0.4	3.11
	2011: Chick sex $*$ chick trt.	-0.5	0.034	5.5 ± 2.5	2.20
Aggression rate					
	Chick treatment (+ year)	-5.1	0.015	6.4 ± 2.3	2.76
	Rank (+ year)			6.4 ± 2.3	2.76
	Chick trt. $*$ rank (B chicks)	-1.0	0.073	10.0 ± 5.4	1.86
	Chick trt. $*$ rank (C chicks)		0.029	12.3 ± 5.4	2.29
	2011: Chick trt.	2.0	0.863	-0.1 ± 0.4	-0.17
	2011: Trt. $*$ rank (B chicks)	-5.4	0.008	1.7 ± 0.6	2.78
	2011: Trt. $*$ rank (B chicks)		0.016	1.6 ± 0.6	2.52

4.4 Discussion

In this study, we have shown that anti-nematode treatment of European shag chicks can alter behavioural interactions within the family that may influence the distribution of resources. Treating chicks increased chick begging and parental provisioning and altered aggression levels of different siblings with variable effects across the two years. Treating parents, on the other hand, had little impact on behaviour in the nest. This is, to our knowledge, the first study to investigate whole-family behavioural impacts of whole-family parasitism, experimentally accounting for expected correlations between parasite burden in chicks and parents. In concert with our anti-parasite treatment experiment, we have described intra-familial behaviours that have not previously been

characterized in the Phalacrocoridae (shags and cormorants). These results illustrate the importance of behaviour to how the effects of anti-nematode treatment, and by extension the costs of parasitism, are distributed among family members.

4.4.1 Significance of behaviours in the nest

Chicks were never fed without begging first, indicating that begging was necessary to obtain food. However, there appears to be a considerable element of parental control of provisioning because parents responded to less than half of all begging bouts. Begging is probably at least in part a reaction to internal state, e.g. hunger, as the initiation of begging frequently seemed not to be triggered by external events. This contrasts with the passerine model systems commonly used to address behavioural questions relating to parent–offspring conflict, in which the parent's arrival triggers begging (e.g. red-winged blackbirds of Forbes et al., canaries of Kilner et al. and house sparrows of Mock et al.). In addition, it was rare for more than one shag chick to beg simultaneously, whereas in the common model systems, the whole brood tends to beg at once. There was substantial variability around the relationship between begging and provisioning, suggesting a complex signal–response dynamic that may also be influenced by other factors, for example previous feeding history.

Feeding duration was a good proxy for how much food chicks obtained, measured as their change in mass. This result provides a tractable and calibrated metric of food delivery, which can be used in future studies of parental provisioning behaviour to minimize disturbance to the nest. The mean feed size of 25g indicates that a parent might make on average four feeds from a single foraging trip (average food load returned to the nest 106g in 1991; Wanless et al. (1993b)). This also suggests that parents do not transfer their whole food load in one feed, although it is not yet clear how much control parents or chicks have over food delivery during a feed. Interestingly, in 2010, chicks were fed less for a given amount of begging than in 2011. 2010 was a more productive year than 2011, which suggests that chicks could afford a greater investment of energy per feed they received in the better year. This accords with low seabird productivity in the North Sea being associated with low energetic profitability of prey fish (Wanless et al., 2005).

Aggression between siblings was not obviously related to begging or feeding, so may not be an important part of resource distribution in this species. Biting did not appear to be damaging to the victim, the victim did not display any obvious submis-

sive behaviour (as there is in the related boobies (Sulidae); Valderrábano-Ibarra et al. (2007)), nor did aggression appear to prevent chicks moving around the nest or begging. It may be that any hierarchies that do control access to food have already been established at the age at which we filmed; in blue-footed boobies, this occurs in the first third of a chick's life (Valderrábano-Ibarra et al., 2007), when shag chicks are too small to be reliably observed. Sibling competition may also be partly played out in begging, which in some species involves an element of scramble competition such that some components of begging displays convey information on chick condition, with offspring in better condition giving a stronger signal (in contrast to the honest signalling theory) (Mock & Parker, 1997; Royle et al., 2002; Parker et al., 2002; Mock et al., 2011). In that case, direct aggression could be of less consequence in sibling competition for access to food than begging. In shags, nothing is known of the function of begging, and neither our observations nor our treatment effects (see below) allow us to differentiate between the two paradigms.

Another aspect of intra-familial communication that is intersting to consider here is the possibility of sibling negotiation (Roulin, 2002). This hypothesis posits that siblings communicate with one another, in between begging to parents, to assess the needs of the whole brood and enable each individual to invest optimally in begging when it occurs. In this way, it is suggested that siblings which would beg at an insufficient level to be fed can postpone their energetic investment in begging until a time when they are more likely to be rewarded for their effort (Johnstone & Roulin, 2003). Much of the empirical work on sibling negotiation has been carried out on barn owls *Tyto alba*, where chicks' vocalizations while parents are absent affect their siblings' begging strategy when parents return with food (Roulin, 2004; Dreiss et al., 2010). It is conceivable that, in shags, aggression between begging bouts serves a negotiating function: if aggression (or indeed other interactions, for example calls) allows chicks to assess their competitors' likely begging levels, it may be decided before a begging bout which sibling is most likely to profit from begging, so that only one chick need beg at one time. It would be rewarding to investigate siblings negotiation further in the shag system, both to better understand this system and to broaden the range of species in which the hypothesis has been applied.

The shag begging framework provides an informative contrast to that of the passerines that are more often used in studies of parent–offspring conflict. Because shag chicks beg singly and not in response to obvious external stimuli, unlike the model passerines, a shag parent's provisioning decision may be very different to the passerine

parents' decisions. A shag must predominantly decide whether or not to respond to a begging chick, presumably using information it has previously gained from other begging chicks, while in passerines, parents are choosing which chick to give a particular food delivery to, weighing up concurrent information from all chicks. This suggests that the selection pressures on both chick signalling and parents' responses to chick signals may differ substantially in shags from the systems on which many theoretical models are built, which thus calls for care in interpreting our treatment effects on these behaviours. These differences may also partly explain why our findings do not reflect previous findings from passerines showing that parents provision parasitized broods at higher rates (e.g. Christe et al., 1996; Hurtrez-Boussès et al., 1998). Nonetheless, begging in shags is a signal that parents respond to, which indicates that it conveys honest information and is thus presumably costly (Godfray, 1995). Parasite-induced changes to either chick signalling effort or parents' responsiveness could, therefore, have fitness implications for both the parents and the chicks.

4.4.2 Anti-parasite treatment effects on behaviour

Chick begging increased treatment in 2010, but not in 2011. This is in keeping with the effects of treatment on chick growth rate also being more substantial in 2010 than 2011 (chapter 2). As expected, chick treatment affected parental provisioning in a similar way, although here siblings of different sex experienced different effects: in 2010, all drug-treated chicks were fed more, whereas in 2011 this benefit only came to sons, not to daughters. Sons grow faster to reach a larger adult size than daughters and their survival benefits more from anti-nematode treatment of parents (Daunt et al., 2001b; Reed et al., 2008), suggesting they may be more expensive for parents to rear. If so, parents may gain more from investing surplus resources primarily in sons (Clutton-Brock et al., 1981), with daughters only benefitting in the more productive of our years, 2010 (Newell et al., 2011). However, we found no evidence of greater provisioning effort to male-biased broods which would support this hypothesis. Instead, the sexes may be differently impacted by parasites, rather than differing in their cost to parents, although we found no evidence for this in its impacts on chick growth rate. Although chick treatment had no overall effect on parental provisioning in 2011, we have shown that it did benefit parents' condition (chapter 3). In chapter 5, we investigate whether this effect on condition might instead be caused by chick treatment altering parents' foraging effort during chick-rearing.

Chick rank did not influence the overall impact of treatment on begging or provisioning, despite rank being a key determinant of the impact of treatment on chick growth rate (only last-hatched chicks respond to treatment; chapter 2). Instead, rank influenced how aggression changed with treatment, but contrary to expectation it was not the last-hatched chick that was most heavily impacted. Older siblings' aggression could affect the last-hatched chick's growth rate by altering its access to food without altering its aggression, although we found no direct connection between aggression and feeding rate to support this. In addition, while increased aggression could reflect dominance, keeping subordinate siblings in their place in the hierarchy (as in Valderrábano-Ibarra et al., 2007), it could also indicate the opposite, with subordinate chicks doing all they can so as not to be completely excluded from the family dynamic (as in Drummond et al., 2003).

Our two years of data provide an indication that anti-nematode treatment of chicks might not always affect behaviour in the same way, as we have shown of its impacts on gorwth rate (chapter 2). We found qualitative differences between years, although we acknowledge that with only two years of data our ability to explore interannual variation is limited. It should also be noted that we were not able to quantify the effect of treatment on worm burden throughout this experiment, and inter-annual variation could depend on differences in initial burdens or the extent of their reduction by treatment as well as on broader environmental differences. We expected the biggest inter-annual difference in treatment effects for last-hatched chicks (chapter 2). Although we found no evidence for such a rank difference overall, an analysis of only C chicks (results not shown) indicated that treatment increased their begging in 2010, when treatment decreased their growth rate, while in 2011, neither begging nor growth rate were strongly affected by treatment. This observation highlights the difficulty in understanding the function of begging in shags, which our experimental design does not allow us to tease apart. Greater begging effort being associated with lower growth rate initially suggests that begging might signal need, but on the other hand anti-nematode treatment increasing begging would suggest that begging signals quality. This, in turn, limits our understanding of how treatment-related changes to these behaviours might impact on chicks' resource acquisistion and hence fitness.

Anti-nematode treatment of parents did not affect any aspect of behaviour in the nest. Our prediction of treatment increasing provisioning was not upheld, perhaps because treated parents might allocate the extra resources to themselves instead of to their chicks. It may be that in this species it is more profitable for parents to invest in their

own condition before the winter, buffering themselves and their subsequent breeding success against environmental stochasticity during the non-breeding season (Frederiksen et al., 2008). We investigate in chapter 5 whether this hypothesis is supported by treatment effect on parents' foraging effort.

Overall, the complexity of our results may be explained in part by two other aspects of chick signalling and parent provisioning in the shag. Firstly, parents may use other signals or cues from chicks than the postural traits we measured, and parasitism could alter different signal components in different ways. For example, Romano et al. (2011) found that immune stimulation of barn swallow chicks increased begging but decreased gape coloration and, perhaps consequently, did not affect parental provisioning. In particular, shag chicks made prominent vocalizations when begging (Snow, 1963) which we were not able to quantify. Secondly, we examined behaviour mainly in relation to the distribution of food, which may not be the only resource over which family members are in conflict. In shags, homeothermy develops slowly (at least into the third week of life; Moe et al. (2004b)), so chicks demand warmth from their parents (Ostnes & Bech, 1997). This may be expensive for parents to provide (Green et al., 2013) and thus a source of conflict, and indeed ectoparasitic infection of great tit parents has been shown to reduce their brooding time (Gallizzi et al., 2008a).

In summary, this study shows that anti-nematode treatment can alter chick and parent behaviour in the nest, indicating that parasitism may play a role in the behavioural interactions between family members. The differences between our two study years in the impact of treatment on begging and provisioning are in keeping with (although do not directly correspond to) interannual differences in the impact of treatment on growth rate (chapters 2 and 3). This suggests that the effect of parasitism on behaviour varies with prevailing environmental conditions, altering chicks' social environment and thus the outcome of intrinsic asymmetries in parasite impacts (Forbes, 2011). This contrasts with a simpler scenario where interannual differences in the effects of anti-parasite treatment on growth are only a function of variation in resource availability. Our results indicate that the behavioural impacts of parasitism can be complex and vary with intrinsic differences between chicks, such as sex and rank, in addition to external conditions that differ between years. However, it is difficult to draw conclusions about how the effects of treatment on behaviour might impact on fitness given that the relationship between these behaviours and growth rate is unclear in this system. Nonetheless, this study is a valuable examination of the impact of anti-parasite treatment on behaviour across the whole family since studies of the effect of parasitism on

both parent and chick behaviour are rare. Furthermore, our experimental design teases apart potential correlations between chick and parent parasite burden, in contrast to many previous studies (e.g. Tripet & Richner, 1999; Bize et al., 2004; Gallizzi et al., 2008a), and thus indicates that chick parasitism, irrespective of parent parasitism, may be important in influencing behaviour in the nest. This illustrates an important mechanism by which parasites can influence host development and breeding success.

CHAPTER 5

Parasitism in offspring alters parents' overwinter foraging and future breeding

5.1 Introduction

The trade-off between current and future reproduction is a central tenet of evolutionary ecological theory (Stearns, 1992; Davies et al., 2012). For an organism faced with limited resources, increased investment in current reproduction is predicted to come at the cost of reduced investment in future reproductive attempts. A range of empirical studies has demonstrated that parasite infection during reproduction can alter parents' current investment, but there has been little work exploring its longer-term consequences. For example, parasitism in parents can reduce their provisioning and other aspects of parental care, leading to impaired growth or survival of chicks (Gallizzi et al., 2008a; Reed et al., 2008; Knowles et al., 2010a). In altricial species, parents may also be affected by parasite infection of their chicks; several studies have shown that parents may increase their provisioning to parasitized chicks (Christe et al., 1996; Fitze et al., 2004b; Bize et al., 2004), and we have previously demonstrated that chick parasitism can reduce parents' condition (chapter 3). The potential of parasitism to alter the trade-off between current and future reproduction has been theoretically demonstrated (Forbes, 1993), yet few empirical studies examine the impact of parasitism on

parents beyond the current breeding event. Further, parents may be influenced both by their own parasite burden and that of their offspring, but we know of no study that disentangles the relative longer-term impacts of parent and offspring parasitism on parents' fitness. Moreover, to our knowledge, no previous work has examined possible mechanisms of such longer-term effects.

During breeding, the costs of parasitism may not be confined to the host but distributed to other family members (chapter 3). In this way, parents may avoid persistent effects of their own parasite burden by transferring costs to their offspring, but might also be affected by infection in their offspring. Moreover, we expect the parasite burdens of parents and their offspring to be correlated: their biotic environments may overlap substantially, parasites may be directly transmitted between family members, and parents and offspring may share genetic bases for parasite resistance. Previous studies showing effects of offspring parasite burden on parents' subsequent breeding (Richner & Tripet, 1999; Bize et al., 2004; Fitze et al., 2004b) focus on systems involving ectoparasites that can move freely between offpsring and chicks (Tripet & Richner, 1999; Bize et al., 2004; Gallizzi et al., 2008a), where anti-parasite treatment of offspring ectoparasites is likely also to reduce parents' exposure to these parasites. It is, therefore, not possible to fully untangle the relative effects of parasites in different family members. Thus, it is as yet unclear to what extent parasitism in breeding parents or their offspring can drive persistent changes to parents' performance.

If parasitism during a given reproductive attempt can also influence future attempts, its overall impact on parents' fitness may not be fully captured in studies restricted to the breeding season. In addition, while manipulations of chick parasite burdens can affect parents' subsequent breeding success (using ectoparasites: Richner & Tripet, 1999; Bize et al., 2004; Fitze et al., 2004b), the potential intervening changes to parents' performance in the non-breeding period have not, to our knowledge, previously been investigated. Parasite-driven alterations to parents' non-breeding performance could have substantial fitness consequences, especially in temperate species, where individuals may be particularly sensitive to external costs during the winter. Less favourable environmental conditions in winter may lead to increased mortality (e.g. Duriez et al., 2012), and visual foragers may in addition be restricted by shorter daylight hours (e.g. Daunt et al., 2006a). Moreover, an animal's ability to maintain condition through the winter can have consequences for its subsequent breeding success (reviewed in Harrison et al., 2011).

Foraging effort is likely to be a key link both ecologically and evolutionarily in the longer-term effects of parasitism, as well as immediately linking parasitism in chicks with effects in parents. Parasite infection imposes resource costs on the host (Colditz, 2008; Atkinson et al., 2009), which hosts could counteract by altering their foraging behaviour. In European shags *Phalacrocorax aristotelis*, anti-parasite treatment of breeding adults increases the time that mothers spent foraging each day (Reed et al., 2008), suggesting a parasite-mediated constraint on foraging effort. By extension, hosts may be able to compensate at a later opportunity for costs incurred during reproduction through increased foraging effort over a longer period of time. In addition to being ecologically important, altered overwinter foraging effort could modify the evolutionary trade-off between current and future reproduction, which is likely underlain by energetic constraint (Stearns, 1992; Wikelski & Ricklefs, 2001; Hasselquist & Nilsson, 2012).

Because of this energetic constraint, foraging effort has been suggested to be an important trait in which carry-over effects in general might be expressed (Daunt et al., 2006a; Harrison et al., 2011). A carry-over effect is a change to any aspect of individual performance caused by events in a preceding season, mediated by a change to individual condition (Harrison et al., 2011). Such patterns are frequently described in wild systems, but may also be due to other correlative explanations (Harrison et al., 2011): overall phenotypic quality of individuals can give inter-seasonal correlations in performance without a causal link (Daunt et al., 2006a), and density-dependent processes may lead to inter-seasonal population-level effects without any within-individual changes (e.g. Duriez et al., 2012). Understanding inter-seasonal effects is of particular interest in seabirds, a group that includes many threatened taxa, whose wide non-breeding ranges make it difficult to follow individuals throughout the year but is also where much mortality occurs (Harrison et al., 2011). Experimental studies differentiating true carry-over effects from these correlative patterns are rare in birds at large (Harrison et al., 2011; but see Studds & Marra, 2005; Catry et al., 2013), but in mammals, anti-parasite treatment has been shown to have persistent effects on traits such as body condition, reproductive success and motor performance in later life (Stien et al., 2002; Vandegrift et al., 2008; Devevey et al., 2010). Chick parasitism has been shown in blue tits *Cyanistes caeruleus* to reduce parents' return rates in the following breeding season (Richner & Tripet, 1999), in Alpine swifts *Apus melba* to reduce parents' subsequent breeding success (Bize et al., 2004), and in great tits *Parus major* to increase parental dispersal the following year (Fitze et al., 2004b). However, in addition to the

question of whether these effects are driven only by treatment altering chick parasite burden or influenced by correlated treatment effects on parents' parasite burden, it is unknown in birds how such between-year effects are mediated through the intervening seasons.

In this experimental study, we investigate the persistence of impacts of parasitism in the family beyond the breeding season. We use the European shag, a piscivorous seabird in which anti-nematode treatment of both parents and offspring have been shown to affect the outcome of a current reproductive attempt (Reed et al., 2008, 2012; chapters 2 and 3, this thesis). Overwinter foraging is an important fitness trait in the shag: thermoregulation is costly (Grémillet et al., 1998), they are visual predators so cannot forage in darkness (Wanless et al., 1993a; Daunt et al., 2006a; Watanuki et al., 2008), and are thus vulnerable to inclement weather and short days during winter in our North Sea study population (Daunt et al., 2006a; Frederiksen et al., 2008). Lower late winter foraging effort is linked to earlier subsequent breeding (Daunt et al., 2006a), which in turn is associated with greater fledging success and recruitment of chicks (Potts et al., 1980; Harris et al., 1994; Daunt et al., 1999). Shags' foraging behaviour can be recorded throughout the year using miniaturized data loggers deployed on the bird (e.g. Daunt et al., 2006a, 2007a), and anti-nematode treatment of parents has previously been shown to increase mothers' foraging during chick-rearing and overwinter (Reed et al., 2008, unpubl. data). The impact of chick parasitism on parents' foraging behaviour, on the other hand, has not yet been examined. This is likely to be an important aspect of the role of chick parasitism in parents' fitness, both in terms of the current breeding attempt and overwinter performance, as we have previously shown that parents' condition during chick-rearing, an indicator of their investment in a current brood, is more strongly affected by anti-nematode treatment of their chicks than of parents themselves (chapter 3).

Here, we use anti-nematode treatment of parents and/or chicks to test the influence of both chick and parent parasitism on parents' subsequent breeding. Our fully factorial treatment design addresses the possibility of correlations between parent and chick parasite burdens. In addition, we investigate whether the effect of chick treatment on parents is associated with a change in foraging effort during chick-rearing and whether it carries over to affect winter foraging. We predict that anti-nematode treatment of chicks should decrease the amount of resources that parents invest during the breeding season, enabling them to maintain better condition. This may last through the winter if chick treatment reduces parents' foraging requirements such that they meet their en-

ergy needs more easily, and hence improve subsequent breeding success. In addition, our experimental approach allows us to separate a true carry-over effect on overwinter foraging from other correlational explanations.

5.2 Methods

This work was carried out in 2011–2012 on the breeding shag population on the Isle of May in south-east Scotland (56°11 N, 2°33 W). The present study extends an investigation into the distribution of the costs of parasitism among family members (chapters 3 and 4) by examining the longer-term effect of anti-nematode treatment of parents and chicks on subsequent breeding and investigating parental foraging as the mechanism for these effects. The core design of the experiment was anti-nematode treatment of parents and/or their offspring in a two-by-two factorial design (detailed methods in chapter 3). All blood sampling and injection was carried out under Home Office licence (personal licence no. PIL 60/12450, project licence PPL 60/3444), ringing under license from the British Trust for Ornithology, and experiments under a National Nature Reserve research licence from Scottish Natural Heritage (license no. MON/RP/124).

In 2011 (henceforth "initial" year), experimental nests were monitored from the colony-wide onset of laying, and hatch date predicted based on observed lay date (accurate to within two days) and a 35-day incubation period (Potts et al., 1980). We used only nests of 3 eggs, the modal clutch size (Snow, 1960). Experimental nests were assigned to a parental treatment group, drug-treated or control, matched for lay date and colony area. At 3–7 days before predicted hatching, both parents on a nest were injected intramuscularly with either the anithelminthic drug ivermectin (Panomec©by Merial, 1% wt/vol) or a saline control, both at a dose of 0.7mg/kg. Nests were monitored daily as predicted hatch date approached to obtain an accurate hatch date, and hatchlings were marked individually. All experimental nests were assigned to a chick treatment group, randomized but matched according to parent treatment, surviving brood size, hatch date and colony area. When the oldest chick in a brood was 10–12 days old, all chicks in the nest were treated with 0.05ml ivermectin or saline.

At capture, parents were weighed and their head-bill length measured. At this point, we deployed miniaturized data loggers on a subset of control parents (65 out of 72 individuals, 26 nests with loggers deployed on both parents). We used geolocator activity loggers (GLS loggers, British Antarctic Survey, Mk 3, 7 & 19) that record the logger's state as wet or dry every 3 seconds. In shags, immersion time very closely

reflects foraging time as they spend little time on or in the water unless hunting (Daunt et al., 2006b). The devices weighed up to 9g (0.4–0.5% of shag body weight) and were attached with cable ties to a darvic leg ring on the bird. Overwinter deployment of GLS loggers has previously been shown not to affect breeding performance in shags (Daunt et al., 2006a) and in this study, carrying a logger did not affect a shag's likelihood of returning to breed or breeding timing the following year (across all experimental birds: effect of logger on returning probability, -0.2 ± 0.6, $p = 0.737$; effect of logger on hatch date (model includes intial hatch date), -1.1 ± 1.9 days, $p = 0.559$). Parents were recaptured when chicks were aged c.30 days, weighed again, and the logger exchanged for another that stayed on through the winter. Exchanging the logger anticipated the possibility of the bird not returning to breed or not being caught and thus losing breeding season data, as these devices are archival and must be retrieved to obtain the data (9 birds in our case; see section 5.2.1 below).

In the breeding season following the experiment (2012, henceforth "subsequent" year), returning loggered birds were recaptured during chick-rearing and the logger removed. We also investigated the effect of chick and parent treatment on four features of parents' subsequent breeding: whether breeding was attempted; whether the initial pair remained intact (mate retention), as divorce is associated with reduced reproductive success in many seabirds and may also be associated with phenology (Bried & Jouventin, 2002; Naves et al., 2006); hatch date, as earlier laying is associated with increased success in this species (Potts et al., 1980; Harris et al., 1994; Daunt et al., 1999); and reproductive success as number of chicks fledged. Hatch date was observed directly in a minority of nests, and otherwise calculated from wing length when chicks are ringed. Of the 125 ringed adults across our treatment groups, 15 did not return to breed (88% return rate, which accords with the long-term average of $\sim$85%).

5.2.1 Statistical analysis

Across the year, foraging data showed a complex non-linear pattern (fig. 5.1) and a single model encompassing the whole year would have been difficult to interpret with regard to treatment effects. Therefore, we chose to divide the data into biologically relevant time periods and examine chick-rearing (to complete chick independence) separately from the overwinter period, fitting linear models to each part. Our basic unit of measurement for foraging effort was total daily foraging time as hours per day. We excluded data from deployment and retrieval days when the logger was not on the bird for the whole day.

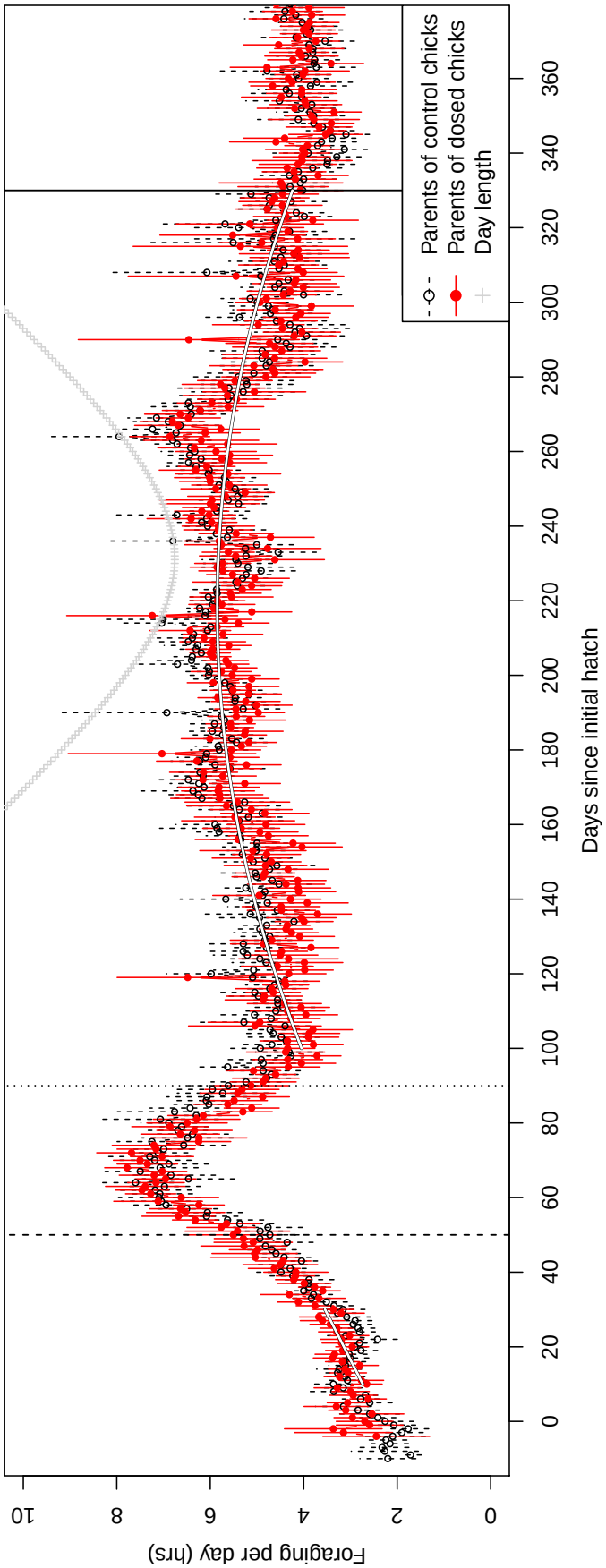


Figure 5.1: Daily foraging time throughout the year, in relation to chick age, for parents of control chicks (black open symbols, dashed error bars) and drug-treated chicks (red solid symbols and solid error bars). Points show means and 95% confidence intervals each day for the two treatment groups. The pale grey line shows mean day length at a given chick age (accounting for the spread in hatch dates and hence different day lengths at the same chick age) to illustrate the constraint of winter daylight on available foraging time. Key events in reproduction are given: the long-dashed line shows fledging at age 50 days, the small-dashed line complete chick independence at 90 days, and the solid black line precisely 1 year after initial laying. The white fitted lines show the periods analysed: early chick-rearing (chick age 10–30 days) was modelled as a linear relationship, and over-winter using a quadratic fit (see section 5.2.1).

5.2.1.1 Chick-rearing

The chick-rearing analysis period lasted from chick treatment at age 10 days, beyond fledging at ∼50 days, to complete chick independence at age ∼90 days, when parents stop provisioning (Snow, 1960). Within chick-rearing, we looked separately at the linear chick growth phase in early chick-rearing (age 10 to 30 days), which corresponded to a linear change in parental foraging (in this period, linear fit compared to quadratic, $\Delta\text{AIC} = -2.1$) and to the end of the period over which parental mass change was measured (mean chick age at parent recapture 29.1 ± 0.3 days; chapter 3). During this period, we investigated two variables: how daily foraging effort (hours per day) changed with chick age and total foraging time across the period. Total foraging was a proxy for total parental investment during the period in which mass change was measured, and was derived for each bird by integrating a linear regression fitted across this time span for each individual. This accounted for gaps in the data due to birds being uncatchable at the intended time (gaps in data for 9 out of 50 birds).

In the late chick-rearing period, from 30 days to independence at 90 days, parental foraging was strongly non-linear. We used three complementary modelling approaches: a generalized additive mixed effects model (GAMM), a piecewise regression, and a generalized additive model (GAM). The GAMM closely fitted the non-linear pattern and allowed fitting random effects to account for repeated sampling of individuals, but only gave descriptive differences between treatment groups. The piecewise regression quantified differences between the groups by approximating the non-linear fit as three linear segments, but did not allow fitting random effects. We therefore fitted this model to the mean value for each treatment group at each day of chick age. The GAMM fit informed our starting values for the iterative model fitting procedure for the piecewise regression. The GAM combined both approaches, closely fitting the non-linear relationship but without random effects, and could be compared using likelihood ratio tests to assess the significance of the treatment effect (not possible with GAMMs). The treatment groups differed in shape such that only the early chick-rearing period (10–30 days) could be directly compared between groups using linear models (see section 5.3.2 below), supporting our separate focus on early chick-rearing.

5.2.1.2 Overwinter

We defined the overwinter analysis period as beginning at chick age 100 days, to ensure that supplementary provisioning had completely stopped (Snow, 1960), and ending

one year after the earliest initial lay date, approximating the start of predicted subsequent laying. We modelled overwinter daily foraging as a quadratic relationship with time, including a linear date term. This fitted the data substantially better than a linear model ($\Delta\text{AIC} = -761$) and agrees with previous findings that overwinter foraging effort increases towards the winter solstice and then declines (Daunt et al., 2006a). All interactions presented were fitted only to the linear date term, not the quadratic term. Fitting interactions to the quadratic instead of the linear time term gave no greater explanatory power (no improvements to AIC) and was less parsimonious. Further, in most cases, fitting the interaction to both the linear and quadratic terms worsened the fit of the models and reduced the significance of the interactions, suggesting that the linear and quadratic interactions are explaining much of the same variation in the data. In addition to daily foraging totals through time, we used individuals' total foraging time per month in late winter to investigate the association between foraging effort and timing of breeding (as demonstrated by Daunt et al., 2006a).

For both chick-rearing and overwinter foraging, we modelled the change in foraging effort through time using chick age as our temporal explanatory variable. Chick age is more biologically informative than absolute calendar date and provided a better fit overall in all time periods (compared to Julian date with or without hatch date, chick age with hatch date $\Delta\text{AIC} \leq -2$ for early chick-rearing, whole chick-rearing and overwinter).

5.2.1.3 Subsequent breeding

In the subsequent breeding season (the year following treatment), we examined the impact of both chick and parent treatment on parents' breeding. This analysis used data for all experimental birds, including drug-treated parents and control parents that did not carry loggers overwinter (sample sizes in table 5.1). Whether breeding was attempted (nest built) and mate retention were modelled as binary variables with binomial errors, timing of breeding as the absolute shift in hatch date from initial hatch date with normal errors, and breeding success as the number of chicks fledged with poisson errors.

5.2.1.4 Analysis details

We tested for chick treatment as a main effect and whether treatment effects differed between mothers and fathers or with initial timing of breeding (hatch date). In addition,

Table 5.1: Sample sizes used in the analysis of logger data during chick-rearing and overwinter, and testing the effects of treatment on breeding in the subsequent year. Subsequent breeding sample sizes are shown as "birds that returned (birds that did not return)".

	Control chicks	**Drug-treated Chicks**
Loggers:		
Chick-rearing	24	25
Overwinter	21	16
Subsequent breeding:		
Control parents	30 (3)	24 (4)
Drug-treated parents	27 (3)	24 (4)

for subsequent breeding, we tested for parent treatment as a main effect and interacting with chick treatment, sex or timing of breeding. Both sex and phenology may be linked to foraging strategy in seabirds (Lewis et al., 2002; Daunt et al., 2006a, 2007b) and the impact of anti-nematode treatment (Reed et al., 2008). In our study year, chick treatment improves parents' condition during chick-rearing for early breeders but decreases parent condition for late breeders (chapter 3, fig. 3.2). Hatch date was fitted as a continuous variable. We also tested initial hatch date as a two-level factor (before or after median), in case pre-median nesters differed qualitatively from post-median nesters (as suggested by the effect of chick treatment on parent mass change), but this did not qualitatively affect the patterns. In all foraging models, we tested for an effect of chick treatment both as a main effect and in interaction with chick age, to test whether treatment effect changed over time.

All analysis was done in R 2.15.1 (R Development Core Team, 2011). We used linear mixed effects models (LMMs) fitted in the package nlme (Pinheiro et al., 2012), generalized linear mixed effects models (GLMMs) in package lme4 (Bates et al., 2011), GAM(M)s fitted in the package mgcv (Wood, 2011), and piecewise regression in the package segmented (Muggeo, 2008). All models (apart from GAM and piecewise regression) were fitted with initial nest as a random factor to account for non-independence of mates, both during chick-rearing and afterwards as both members of pairs that remained intact bred at the same time in both years. Of a total of 62 pairs investigated where both members were ringed (both control and drug-treated

parents), 20 (32%) bred together again in the subsequent year. In addition, all models of foraging in relation to time included bird ID as a random factor, nested within nest, to account for repeated sampling of individuals through time. Birds raised similar-sized initial broods, which could affect foraging effort, in both treatment groups for birds carrying loggers ($\chi^2 = 1.53$, d.f.$= 2$, $p = 0.465$), and in all four treatment groups across all experimental birds ($\chi^2 = 9.34$, d.f.$= 6$, $p = 0.154$).

In this chapter, we restrict our analysis to the same subset of nests as chapter 3 (see there for details), excluding four nests that were second breeding attempts, one consisting of a brother–sister pair, and three that were anomalously late. We excluded a further two birds from our analyses of early chick-rearing that had less than 10 days' data, which led to unreliable slope estimates in this restricted time period. Of the parents carrying loggers over winter, 7 did not return to breed in the subsequent year, 2 could not be caught, 1 logger had become detached and lost during the winter, and 2 loggers returned corrupt data that could not be analysed. Final sample sizes were: carrying early chick-rearing loggers, 49 birds in 28 nests; carrying whole chick-rearing and overwinter loggers, 37 birds in 26 nests; for subsequent breeding (all experimental parents), 105 returned birds from 60 initial nests (table 5.1), with hatch date available for 91 birds in 55 nests.

5.3 Results

Anti-nematode treatment of chicks had little effect on parents' foraging effort during chick-rearing but reduced it overwinter, culminating in parents of drug-treated chicks breeding earlier in the following year.

Overall, foraging time ranged from 0 to over 10 hours per day, with a mean of 3.10 ± 0.03 hrs/day in early chick-rearing (average day length of 17.5 hrs), and a mean of 5.95 ± 0.04 hrs/day in December (average day length of 6.9 hrs) (fig. 5.1). Individuals varied in their daily foraging time (as inter-quartile range) by $\sim 15\%$ of available daylight each day (13% after fledging in first week of August, 16% in week around winter solstice). Across the year, foraging time followed a non-linear pattern (fig. 5.1). Foraging increased linearly through early chick-rearing, then increased sharply before chicks left the nest at age ~ 50 days to a peak around chick age 67 days. Foraging then declined sharply as chicks reached complete independence at ~ 90 days. From chick independence to laying in the spring, daily foraging time increased towards the winter solstice and decreased after it, constrained by day length for circa one month either side of the solstice and peaking before and after this constraint.

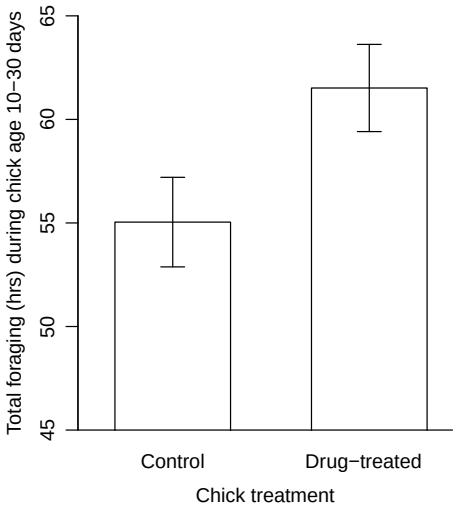


Figure 5.2: The total time that parents spent foraging in our early chick-rearing period (chick age 10–30 days) for parents of control and drug-treated chicks. Bars show means ± 1 standard error.

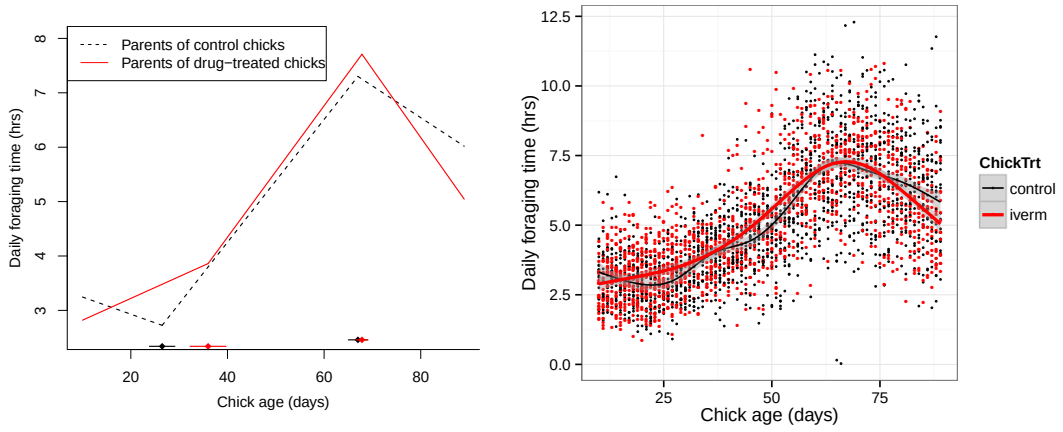
5.3.1 Early chick-rearing

Chick treatment did not significantly alter their parents' total foraging across the early chick-rearing period, although there was weak support for parents of anti-nematode treated chicks foraging more than parents of control chicks ($\Delta\text{AIC} = -1.9$ from intercept-only model; total foraging during chick age 10–30 days, treatment effect size 6.1 ± 3.1 hrs (equivalent to c. 2 days' foraging) , $t = 1.99$, $p = 0.059$; fig. 5.2), irrespective of parents' sex or phenology (interaction models $\Delta\text{AIC} > -0.9$ from main treatment effect model). Parents of drug-treated chicks foraged slightly more each day as their chicks got older, whereas parents of control chicks reduced their foraging slightly with chick age (treatment * chick age model $\Delta\text{AIC} = -18.7$ from main effects only; interaction parameter 0.04 ± 0.01 hrs/day, $t = 4.44$, $p < 0.001$; drug-treated group slope 2.0 ± 0.4 mins/day, control group slope -0.7 ± 0.4 mins/day; fig. 5.3a).

Total foraging time did not predict parents' mass change across the experimental period (total foraging time as single main effect, $\Delta\text{AIC} = -0.8$ from intercept-only model).

5.3.2 Late chick-rearing to independence

On average, parents of drug-treated chicks increased their pre-fledging foraging effort 9 days later than parents of control chicks, but both groups reached their peak for-



(a) Piecewise regression

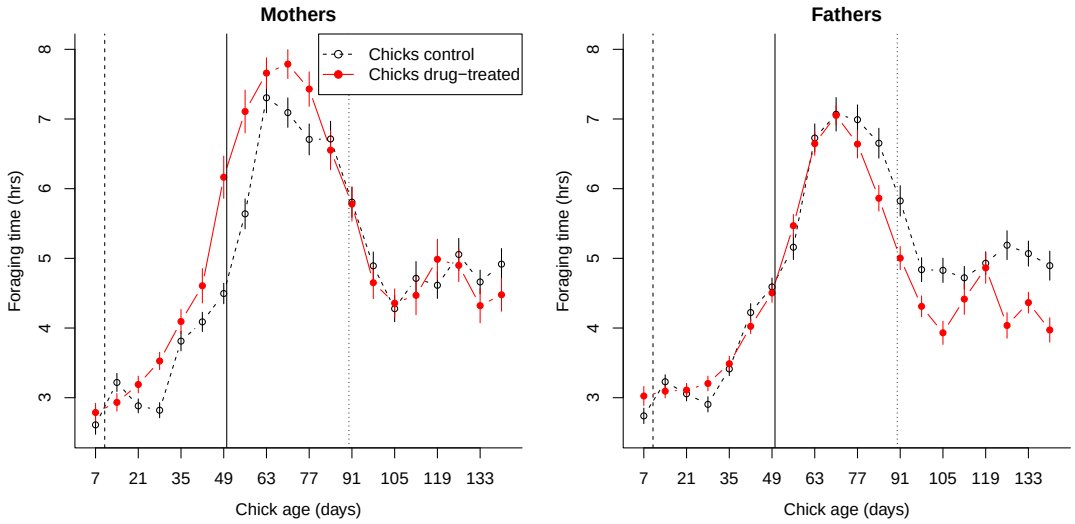
(b) GAMM

Figure 5.3: Two alternative approaches to investigating non-linear patterns in parental foraging effort across the whole chick-rearing period (from chick treatment at 10 days to complete independence at 90 days). These graphs include the early chick-rearing period (chick age 10–30 days) which was modelled separately as a linear relationship. Fig. 5.3a shows a piecewise regression and fig. 5.3b a GAMM. Fitted lines are given for parents of control (black) and treated (red) chicks, and for the piecewise regression, estimates and 95% confidence intervals of the break points (see table 5.2) are shown by the x-axis.

aging at the same time (chick age 67 days) (table 5.2, fig. 5.3). The groups differed significantly in their non-linear foraging pattern in this period (F-test comparing GAM with different smoothers for each treatment group to GAM for all birds combined, $p < 0.001$). During this period, chick treatment did not affect the total time that parents spent foraging (sum per bird from chick age 40 to 90 days, $\Delta\text{AIC} = 0.9$ from intercept-only model).

No single model could formally test whether treatment responses in this non-linear pattern differed according to parent sex or phenology. However, there was a qualitative suggestion that treatment increased the total amount of foraging in this post-fledging peak for mothers but made less difference to fathers (fig. 5.4a). In addition, treatment appeared to make little difference to post-fledging foraging for early nesters, whereas for later nesters, parents of drug-treated chicks increased their foraging earlier and to a higher peak than parents of control chicks (fig. 5.4b).

(a) Effect of sex



(b) Effect of hatch date

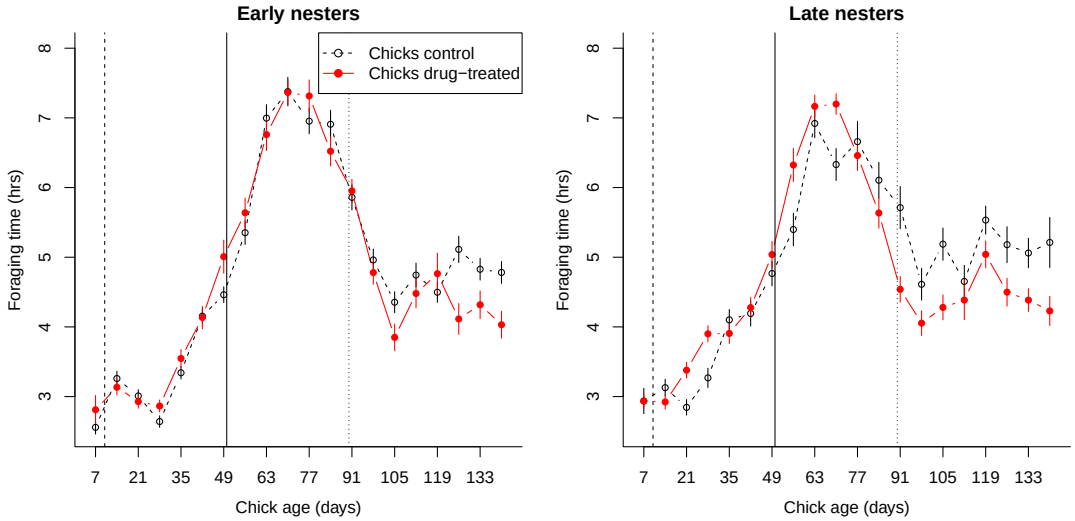


Figure 5.4: The effect of chick treatment on the non-linear pattern of daily foraging time throughout the chick-rearing period, illustrating qualitative differences between parents of different sex (upper panels) and phenology (lower panels). The plot shows mean daily foraging time ± 1 standard error per week of chick age. The long-dashed line at 10 days shows the point of chick treatment, the solid line at 50 days shows fledging and the short-dashed line at 90 days complete chick independence, i.e. the end of supplmentary feeding by parents.

Table 5.2: The piecewise regression fit for daily parental foraging times through the chick-rearing period. Parameter estimates are shown with lower and upper 95% confidence intervals, and parameters that differed significantly between the groups are shown in bold. Slopes are the change in daily foraging time per day and break points are for chick age in days. The model was fit to the means per day of chick age for control and drug-treated groups separately.

	Control chicks			Drug-treated chicks		
Parameter	**Estimate**	**Lo. C.I.**	**Up. C.I.**	**Estimate**	**Lo. C.I.**	**Up. C.I.**
Slope to 1st break	**−0.031**	−0.064	0.001	**0.040**	0.025	0.055
1st break point	**26.5**	23.9	29.1	**36.0**	32.3	39.7
Slope 1st–2nd break	0.113	0.104	0.122	0.121	0.110	0.132
2nd break point	67.0	65.0	69.1	67.8	66.6	69.1
Slope from 2nd break	**−0.059**	−0.081	−0.036	**−0.126**	−0.145	−0.107

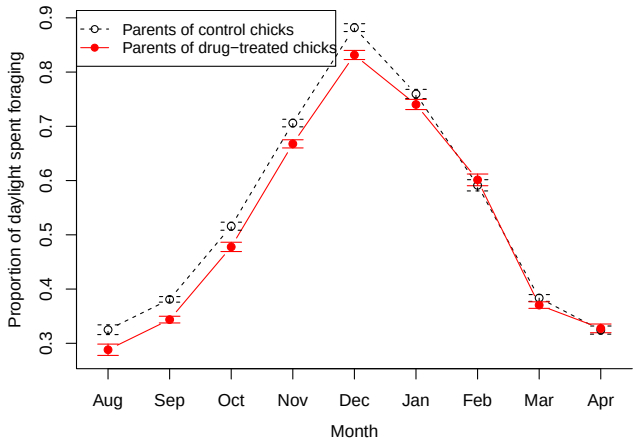


Figure 5.5: Average daily foraging time each month for parents of control (black open symbols and dashed line) and drug-treated (red solid symbols and line) chicks as a proportion of available daylight (day length sunrise–sunset). Points and error bars show the mean ± 1 standard error.

5.3.3 Overwinter

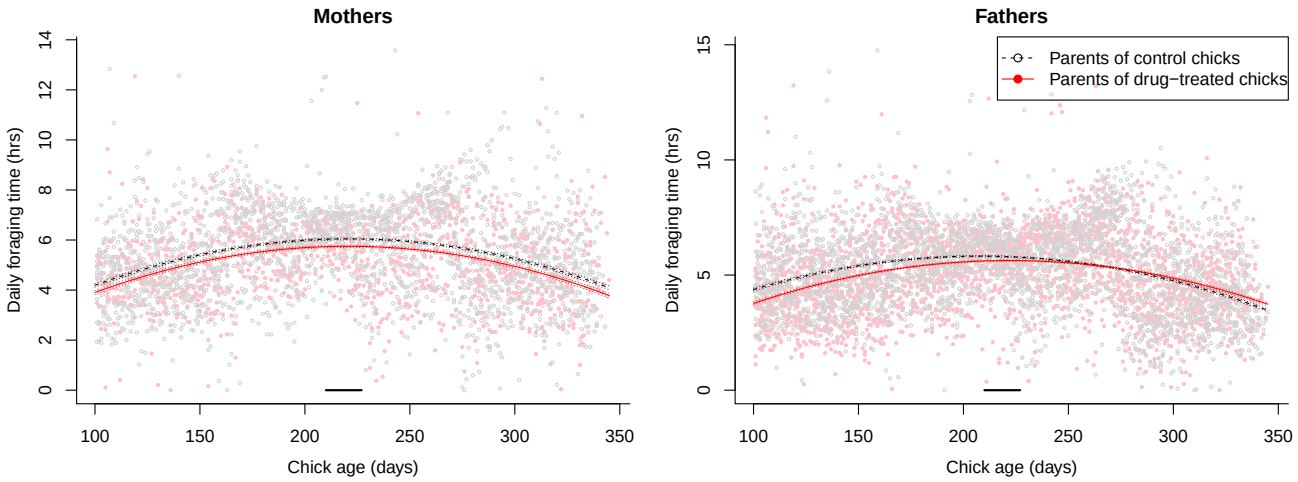
Through the winter, parents of drug-treated chicks foraged less per day than parents of control chicks (table 5.3). This amounted to an average total of 43.0 hours less during the autumn and winter, equivalent to c.8 days' foraging (total foraging September–February: parents of drug-treated chicks, 969.6 ± 22.3 hrs; parents of control chicks, 1012.6 ± 29.6 hrs). This pattern was mirrored in the proportion of available daylight that parents spent foraging, under the constraint of short days in midwinter (fig. 5.5). The effect of chick treatment differed between mothers and fathers and between early and late initial nesters (table 5.3). Mothers of drug-treated chicks foraged less throughout the winter than mothers of control chicks, whereas fathers' foraging treatment was only reduced by chick treatment before midwinter (fig. 5.6a). Similarly, chick treatment reduced parents' foraging only up to midwinter for birds that initially nested before the median, whereas for post-median nesters, chick treatment reduced foraging throughout the overwinter period (fig. 5.6b).

Total foraging in February was linked to subsequent hatch date, with parents that foraged less throughout February breeding earlier (fig. 5.7). The relationship was weakly supported among all parents carrying loggers ($\Delta\text{AIC} = -1.9$ from intercept-only; February $\Delta\text{AIC} = -1.2$ from the next-best month) but was significantly stronger for parents of drug-treated chicks than parents of control chicks (subsequent hatch date described by February total foraging * chick treatment, $\Delta\text{AIC} = -4.8$ from February total foraging only; interaction effect size 0.18 ± 0.05 days per hour of foraging, $t = 3.51$, $p = 0.010$; fig. 5.7). However, February foraging did not directly affect breeding success (interaction with chick treatment on number of chicks fledged, $p = 0.944$; as main effect, $p = 0.108$).

5.3.4 Subsequent breeding

We examined the subsequent breeding of all experimental birds (including drug-treated adults and control adults that did not carry loggers). Parents of drug-treated chicks bred almost a week earlier than in the initial year, whereas controls did not alter their breeding timing (chick treatment main effect on shift in hatch date, $\Delta\text{AIC} = -6.8$ from intercept-only model; effect size -6.4 ± 2.1 days, d.f.$= 53$, $t = -2.97$, $p = 0.005$). Mothers and fathers were similarly affected, as were early and late initial nesters (both interactions $\Delta\text{AIC} > -1.0$ from main effect model; fig. 5.8), leading to an overall earlier distribution of subsequent hatch dates for parents of drug-treated chicks (fig. 5.9).

(a) Effect of sex



(b) Effect of hatch date

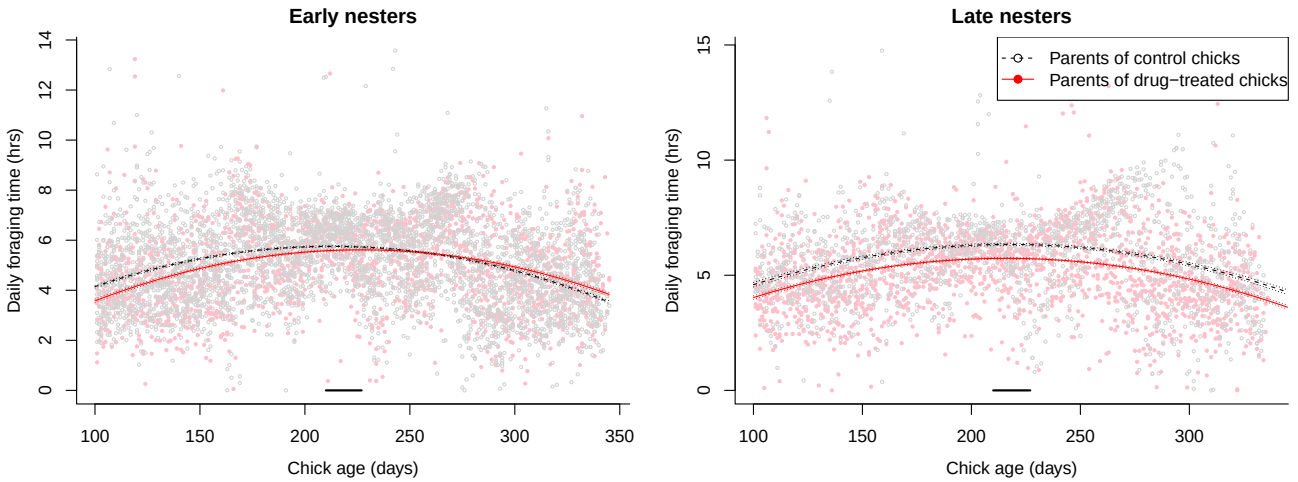


Figure 5.6: The effect of chick treatment on parents' foraging time shown separately for fathers and mothers (upper panels), and for early and late initial nesters (lower panels). For the sake of illustration, we present the effect of initial hatch date by showing birds nesting before the median separately to those nesting after, but the model was fitted to a continuous measure of hatch date. All models were fitted to chick age as the temporal variable; for orientation, the black line around age 210 days denotes the period when the winter solstice (most constrained foraging) fell for all but the two latest birds.

Table 5.3: Fits of models tested to describe the overwinter foraging of adult shags and parameter descriptions for the best fit model. The time variable is chick age and effect sizes are for daily foraging effort in hours per day. ΔAICs are given relative to the underlying pattern of foraging effort in relation to Time^2 + Time (first model) and effect sizes are in minutes of foraging each day. Hatch date refers to initial hatch date and is abbreviated to "H.D.".

Model	**Model ΔAIC**
Time^2 + Time	0
Time^2 + Time + Chick trt.	0.7
Time^2 + Time * Chick trt.	-11.1
Time^2 + Time * Chick trt. * Sex	-26.7
Time^2 + Time * Chick trt. * H.D.	-16.8
Time^2 + Time * Trt. * Sex + Time * Trt * H.D.	-31.5

***Best fit model*:**

Parameter	**Estimate**	**Std. error**	**t-value**	**p-value**
Time * Chick trt. * H.D.	0.0	0.0	-2.57	0.010
Time * Chick trt. * Sex	0.2	0.1	3.36	0.001

Breeding success declined through the season overall (hatch date main effect on no. chicks fledged, $\Delta\text{AIC} = -3.2$ from intercept-only; effect size -0.02 ± 0.01 chicks/day, $z = -2.29$, $p = 0.022$). However, there was no direct effect of chick treatment on breeding success (treatment in addition to or interacting with hatch date, $\Delta\text{AIC} = 2.0$ from hatch date only model).

Anti-nematode treatment of parents themselves did not affect their subsequent lay date (adult treatment in addition to or interacting with chick treatment, $\Delta\text{AIC} > 1.4$ from chick treatment only). Return rate and mate retention were not directly affected by either treatment (as main effects, interacting with each other or with sex or initial hatch date, all $\Delta\text{AIC} < -0.9$ from intercept-only). Mate retention did not influence the effect of chick treatment on phenology (main effect and interacting with treatment and subsequent hatch date,$\Delta\text{AIC} < 1.7$ from treatment-only model).

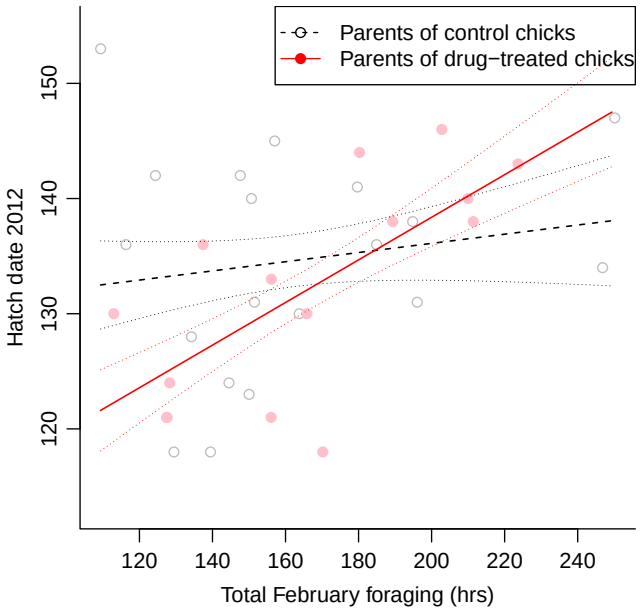


Figure 5.7: The relationship between total foraging in February and subsequent hatch date for parents of drug-treated chicks (red, solid line and symbols) and control chicks (black, open symbols and dashed line). The plot shows the fitted linear relationship with 95% confidence intervals.

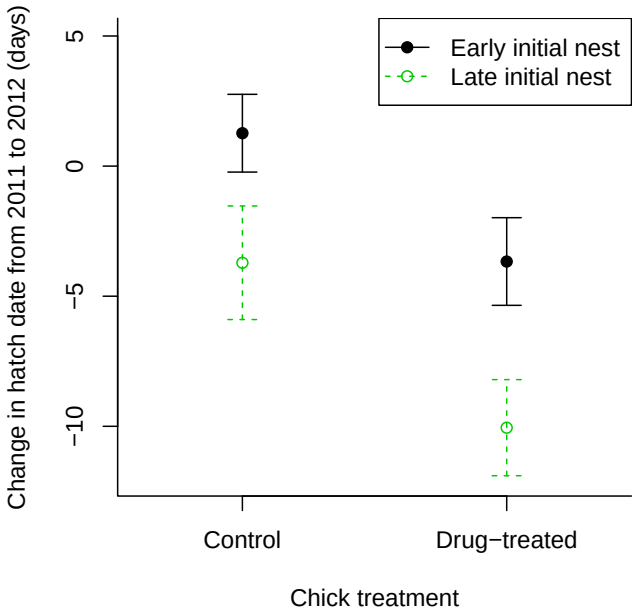


Figure 5.8: The effect of chick treatment on parents' subsequent timing of breeding for those with early initial timing of breeding (black, solid symbols) and late initial breeding (green open symbols and dashed lines). Points show means $\pm$ 1 standard error.

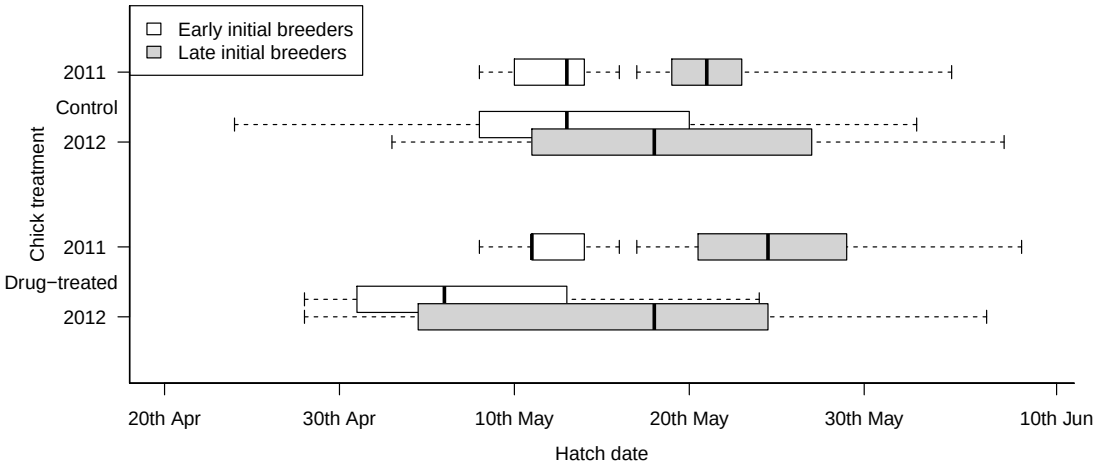


Figure 5.9: The distribution of hatch dates for early initial nesters (white bars) and late initial nesters (grey bars) in the initial year (2011) and subsequent year (2012) for parents of control chicks (upper set of bars) and drug-treated chicks (lower set of bars). Boxes show the interquartile range, the thick line shows the median, and the whiskers show whole range of hatch dates.

5.4 Discussion

In this experimental study, we found that anti-nematode treatment of chicks influenced parents' performance not only when chicks are in the nest but beyond the breeding season, through the winter and to the subsequent breeding season. Although there was little effect of chick treatment on parents' foraging during chick rearing, parents of drug-treated chicks foraged less during the winter and bred earlier in the subsequent year than parents of control chicks with natural nematode prevalence. This study is, to our knowledge, the first experimental investigation of longer-term effects of anti-parasite treatment to disentangle the effects of parent and offspring parasitism, and the first description of a mechanism by which cross-generational effects of parasitism persist beyond the breeding season in birds. In addition, we have demonstrated a true carry-over effect by experimentally excluding other correlational explanations, adding to the currently sparse body of such evidence.

The effects of chick treatment on parents' winter foraging and subsequent breeding are likely to have fitness implications for the parents. Poor winter conditions are associated with changes to important demographic traits including increased mortality, delayed breeding or non-breeding (Aebischer & Wanless, 1992; Frederiksen et al., 2008), and shags are vulnerable to extreme winters as they do not carry large fat reserves and

their foraging opportunities are vulnerable to adverse weather conditions (Grémillet et al., 1998; Daunt et al., 2006a; Frederiksen et al., 2008). The treatment-induced reduction in daily foraging time in winter could, therefore, be of substantial ecological significance. If parents of anti-nematode treated chicks can meet their daily energy demands more quickly, they may be buffered against stochastic reductions in available foraging time and hence more likely to survive the winter and breed successfully the following season. This treatment effect could be particularly beneficial in midwinter, when birds are usually foraging for almost all available daylight (fig. 5.5), and in poor winters when adult mortality can increase six-fold (Frederiksen et al., 2008). The advance in breeding brought about by chick treatment is also likely to be beneficial to parents' fitness as earlier breeders fledged more chicks and have been previously shown to produce more recruits (Harris et al., 1994).

Although the effects of chick treatment on parents' winter foraging and subsequent breeding were well supported, we found no clear effect of chick treatment on parental foraging during chick-rearing. This accords with our previous finding that chick treatment does not affect parental provisioning rates (chapter 4), but leaves unexplained our finding that chick treatment affects parents' mass change during the chick-rearing period (chapter 3). Getting to the bottom of this apparent gap would require further work, perhaps investigating other details of provisioning and foraging such as the energetic value of the food that parents are catching and providing to chicks, which varies between prey species (Wanless et al., 2005). Interestingly, we found no differences between mothers and fathers in the effect of chick treatment on foraging during chick-rearing, suggesting that both sexes are similarly unresponsive to chick need. This contrasts with the effect of parent treatment, which was found in a previous study to increase mothers' foraging but not fathers' (Reed et al., 2008). Similarly, the effect of chick treatment was the same for breeders across the season, unlike its effect on parental condition, which varied with hatch date (chapter 3). This could suggest that parasitism in chicks might influence parental resource allocation in different ways throughout the season, which we unfortunately cannot fully test as our provisioning data came from predominantly early nests with three chicks (chapter 4).

In the late chick-rearing period (age 30–90 days), shag parents rapidly increased their daily foraging effort. This indicates that this period may be of particular biological importance for shags to regain condition for the winter (Daunt et al., 2007a). Parents of drug-treated chicks began this increase later than parents of control chicks, suggesting that they may be in less need after the fast chick growth phase. This inter-

pretation corresponds with parents of control chicks foraging less during early chick-rearing, and is supported by the qualitative observation that late-nesting parents of drug-treated chicks, whose condition declined during chick-rearing (chapter 3), increased their foraging faster than early-nesting parents of drug-treated chicks.

Once chicks were completely independent, parents who had raised drug-treated chicks foraged less per day than parents of control chicks, and this difference persisted until midwinter. This matches our predictions that anti-nematode treatment of chicks could allow parents to maintain condition better through chick-rearing and thus require less subsequent foraging effort. However, given that our chick-rearing data did not support a treatment-induced change to foraging or provisioning (chapter 4), it is unclear why treatment effects on foraging should become apparent after fledging. Perhaps a substantial change to foraging, in response to a change in condition over the chick-rearing period (chapter 3), is only possible once parents' foraging is no longer constrained by alternating shifts providing care at the nest. Although the effect of treatment on foraging appeared to decline through the winter, it still influenced subsequent breeding: parents of treated chicks bred almost a week earlier than parents of control chicks. We did not find a direct effect of chick treatment on breeding success, but early breeding was correlated with greater fledging success overall, which warrants caution in the interpretation of this result. Treatment may only affect phenology, which other factors during the season then link (imperfectly) with success, diluting the effect of treatment; alternatively, treatment may actually have a causal effect on phenology but not on success, and from our data we cannot tease apart these two explanations. We can speculate, though, that if the higher success of earlier breeders (Potts et al., 1980; Harris et al., 1994) is due to more favourable early-season conditions (but see Daunt et al., 1999), chick treatment could potentially carry over even further by again, a year later, enabling parents to enter the winter in better condition. As all individuals in the study population are ringed, the question of such long-term effects would be feasible and valuable to pursue.

Interestingly, chick treatment advanced timing of breeding similarly for both early and late initial nesters, in contrast to its effect on parents' condition during chick-rearing, which was positive for early nesters but negative for late nesters (chapter 3). The longer-term effects of anti-nematode treatment of chicks are thus not simply an extension of its immediate effects, which is important to consider in the evolutionary role that chick parasitism may play in parents' reproductive investment decisions. Parents' responses to parasite infection of chicks are commonly interpreted as a pas-

sive consequence of the costs of infection (e.g. Christe et al., 1996; Bize et al., 2004). However, parents may also respond adaptively to maximize their lifetime reproductive success, strategically reducing investment in a parasitized current brood in favour of future attempts, when parasite burdens may be lower (Forbes, 1993). If passive, we would expect the longer-term consequences of chick infection for parents to extend its immediate effects. If adaptive, on the other hand, individuals might differ in their immediate response to chick parasitism – depending on the value of the current brood and their own residual reproductive value – but achieve the same longer-term impacts, such that negative immediate consequences could have positive longer-term consequences. Our finding of similar long-term effects for groups that differed in their immediate responses suggests that parents' counterintuitive physiological responses to chick treatment in late season may not be purely passive. Testing these two hypotheses fully would require knowledge of parents' lifetime reproductive output (Forbes, 1993), but the trade-off is nonetheless indicated by the effect of offspring treatment on parents' breeding in the following year, as we found (as in Richner & Tripet, 1999).

Notably, anti-nematode treatment of parents themselves did not affect subsequent breeding. Given that parent treatment had little impact on their condition during chick-rearing (chapter 3), we might predict a greater treatment influence after fledging, when parents can no longer transfer costs of their own parasite infections to their chicks. We know of no previous study that takes such transfer into account when examining persistent effects of parents' own parasitism during reproduction. In the shags, it appears that the impacts of parent treatment are borne predominantly by early chick mortality (chapter 3). The negligible effect of treatment on parents themselves also highlights the cost of reproduction for adult shags, given that a single perturbation to this cost can have such persistent and substantial consequences, relative to manipulation of their own nematode burden.

In addition to illustrating an influence of parasitism during reproduction on host ecology beyond the breeding season, our results provide an experimental demonstration that conditions during breeding can carry over to affect individual performance in the non-breeding season and beyond. We found similar treatment effects in the subsequent year across a range of individual breeding performance (indicated by initial timing of breeding) for only part of the population (parents of treated chicks). Our results thus represent a true carry-over effect as we have excluded two factors that may give rise to apparently similar outcomes: overall phenotypic quality of individuals or density-dependent population-level changes (Harrison et al., 2011). This could

suggest a causal link between extrinsic events and individual performance across the annual cycle. In particular, our treatment-induced difference in the relationship between February foraging and timing of breeding suggests that they may be causally linked, rather than good-quality individuals being both more efficient foragers and earlier breeders (Daunt et al., 2006a). However, the direction of causality remains unclear: birds may reduce foraging in order to initiate breeding, or birds may be able to initiate breeding because they require less foraging. Moreover, our results show that foraging effort is a key fitness trait in which carry-over effects might be expressed, and suggest that energetic constraint is indeed a key link between parents' reproductive effort from one year to the next. Resource acquisition is commonly invoked as a mechanism for carry-over effects (Harrison et al., 2011) but this has only been experimentally tested once indirectly (Studds & Marra, 2005).

In summary, we have shown that anti-nematode treatment of nestling shags can affect fitness traits in their parents well beyond the nestling period. Behavioural change is an important aspect of the influence of chick treatment on parents, both immediately and in the longer term. This is the first study to tease apart the effects of parent and offspring parasitism on parents' longer-term performance. Moreover, this is a rare demonstration of an inter-seasonal carry-over effect of offspring parasitism on parents and adds to the currently sparse literature exploring carry-over effects experimentally, showing that a single event (chick treatment) can alter parents' performance (foraging effort) beyond the breeding season into the winter and thence to the subsequent breeding season. By demonstrating a mechanism for persistent cross-generational parasite impacts, our findings elaborate the role that parasitism might play in hosts ecology and life history, and illustrate the potential of chick parasitism to influence parental traits of potential consequence for population processes.

CHAPTER 6

General discussion

In this thesis, I sought to produce a more complete picture of the ecological role that parasites play in hosts' reproductive attempts and hence their implications for the fitness of all family members. This work has used experimental anti-nematode treatment of families of the European shag *Phalacrocorax aristotelis* to extend our current understanding of the interplay between parasitism and family conflict in two main aspects. Firstly, I have shown that anti-nematode treatment can have not only immediate, direct impacts for the host, but that its effects may also be distributed among other family members, and persist beyond the breeding season. Secondly, the way in which treatment affects certain parts of the population vary considerably with extrinsic influences, including environmental variability and social interactions. Therefore, by considering the effects of parasitism only for certain individuals in a family or within a single breeding season, we risk underestimating its consequences for the success of all family members. Altogether, these findings suggest that our current understanding of family conflict and life-history decisions may not fully appreciate the role of parasitism. Moreover, this thesis is a rare in-depth experimental study of the role of parasitism in seabird ecology and life-history, and indicates that parasitism may be an important but thus far overlooked influence on population dynamics in these charismatic and ecologically important taxa.

In this general discussion, I explore the implications of my findings for the field and for the study system. In section 6.1, I address issues that the distribution of costs raises for interpreting previous studies of the role of parasites in host families, and contrast the natural history of the shag/nematode system to previously used host/parasite systems. In section 6.2, I draw lessons from the temporal variation in parasite impacts. I then assess in section 6.3 the ecological and evolutionary implications of the persistence of parasite impacts beyond the breeding season. Lastly, I discuss the implications my findings may have for population processes (section 6.4) and raise some caveats in the interpretation of the benefits of treatment (section 6.5) before concluding with some promising directions for future work (section 6.6). In addition to my experimental findings, I have shown in appendix A that endoscopy is a useful tool for quantifying parasite burdens of chicks in this system.

6.1 Distribution of costs

The core experiment of chapters 3, 4 and 5 examined how the costs of infection are distributed among all family members within a single experimental framework. This is, to my knowledge, the first investigation into the simultaneous impacts of parent and offspring parasitism on all family members, and also unusual in experimentally controlling for the possibility that parasite burdens are non-independent across family members. Moreover, most studies of how parasitism might affect resource allocation in the family examine the effects of parasites on only certain family members. My novel finding that anti-parasite treatment can have more marked impacts for other family members than for the treated individuals, both for parents on chicks and chicks on parents, suggests that our current understanding of the role of parasitism in families may underestimate their influence on the fitness of the family as a whole (i.e. parents' inclusive fitness) and thus their role in host life-history decisions. Indeed, many previous studies have found that the direct costs of parasitism are lower than expected, for example chick parasitism not reducing chick growth or survival (Tripet & Richner, 1997; Szép & Møller, 2000) or parent parasitism not reducing parental condition (Tomas et al., 2007), but may overlook indirect costs.

The distribution of costs that I have described could explain some discrepancies between my findings and what we would predict based on the literature to date. While a multitude of studies across a wide range of species has demonstrated an impact of parasitism on reproductive success (Tompkins et al., 2011), previous research on the

influence of parasites on interactions within the family has focused on a limited range of host species with findings that may not directly apply to host species with different ecology. In particular, the life-history trade-offs facing the passerine hosts that have been the focus of this field may be very different to those of the shag, which is generally longer-lived and smaller-brooded. A shorter-lived host is more likely to increase its current reproductive investment in the presence of parasites to decrease the impact of infection on reproductive success, as its survival probability to breed again may be small (Stearns, 1992). It is conceivable that longer-lived hosts such as the shags may, in contrast, invest preferentially in minimizing impacts on themselves in order to maintain their residual reproductive value. Indeed, whereas passerine studies generally find an increase in parental provisioning to parasitized broods (e.g Christe et al., 1996; Tripet & Richner, 1997; Hurtrez-Boussès et al., 1998), shag parents provisioned naturally-parasitized broods at a lower rate than anti-parasite treated broods (chapter 4). However, compared to other seabirds, the shag has a relatively short breeding lifespan and large clutch size (Weimerskirch, 2002), and it would be interesting to test this hypothesis by examining the effect of parasitism on families of species with even slower life histories.

Another important consideration in interpreting previous studies is the possibility that family members share correlated parasite burdens, which is particularly the case for horizontally transmitted ectoparasites that have been the focus of the majority of studies in this field. My experimental design addressed this possibility by independently treating parents and/or chicks within a single experimental design. Thus, although I was unable to collect sufficient data to test the role in this result of different family members' parasite burdens, the effects of chick parasitism on parents and parent parasitism on chicks are likely to be real indirect effects, with costs distributed among other family members than the treated individual. In contrast, in many of the passerine/ectoparasite systems commonly used, a correlation between chick and parent parasite burden has been explicitly shown (Møller, 1994; Tripet & Richner, 1999; Bize et al., 2004; Fitze et al., 2004b) but is very rarely accounted for in parasite manipulation experiments, such that treatment could lead to altered parasite burdens in untreated individuals. In an exception, Gallizzi et al. (2008a) controlled chick parasitism to demonstrate flea-induced maternal effects on flea resistance in great tit *Parus major* chicks, but that study investigated on pre-hatching maternal investment rather than post-hatching family conflict. Experiments using avian malaria as the parasite eliminate one cause of correlation as there is no direct transmission between family

members. However, in most previous studies, it is not possible to rule out that apparent indirect effects of anti-parasite treatment in other family members could in fact be direct effects of a correlated treatment-induced change in unmanipulated individuals. In further support of my interpretation of these indirect effects, my experimental treatment showed no evidence of a parent–offspring correlation in worm burdens in the shags, given that simultaneous manipulation of parent and chick burden had neither additive nor interacting effects (chapter 3). This complements the indications from dissections that direct infection of chicks with adult worms dislodged from their parents is rare (appendix A), although I was not able to quantify these relationships directly. This could be specifically examined using endoscopy of whole families.

The shags' interactions with family members also differ from passerine systems commonly used in studies of family conflict behaviour. In chapter 4, I examined whether the distribution of parasite costs in a family would be apparent in the behavioural interactions between family members governing the distribution of food in the nest, and demonstrated that parasitism can affect all three aspects of intra-familial behavioural dynamics under investigation: chick begging, parent provisioning and sibling competition. However, while anti-nematode treatment of chicks influenced these behaviours, the changes did not directly correspond with our predictions a priori or based on the results from chapter 3, as I discuss in chapter 4. For example, in 2011, chick treatment in the nests included in the behavioural study increased parents' condition, suggesting that parents might provision treated chicks less, but in fact provisioning rate to dosed broods was greater than to control broods. Moreover, the behavioural impacts of treatment did not directly mirror its impacts on chick growth and survival, nor was there a clear link between provisioning and parental foraging effort (chapter 5). I found no aspect of behaviour that directly explained how impacts of treatment were distributed between family members, indicating that care should be taken in interpreting changes to parental provisioning if parental foraging behaviour or the physiological costs associated with provisioning are not accounted for, and neither are typically measured in parasite manipulation studies. Behavioural change in response to parasitism in the shag are difficult to interpret in the light of previous studies because the behavioural dynamics of resource distribution within the family are very different from those in more commonly studied passerine systems, as I discussed in that chapter. In addition to the patterns of begging being very different, the evolution of begging itself may have been under different pressures in seabirds than in passerines. A substantial cost of begging in passerines is thought to be the risk of alerting predators to the loca-

tion of the nest (Wright & Leonard, 2002). Many seabirds, in contrast, have few nest predators, and shags in particular nest conspicuously on the ground, so in evolutionary terms begging in shags may be less costly than in model systems.

6.2 Variability within and between seasons

An important overarching theme of my findings is that parasite impacts vary considerably with external conditions. The effects of chick anti-parasite treatment differed between years (chapter 2) and, within a season, effects of both parent and chick treatment differed with phenology (chapter 3). In parasite manipulation studies, environmental variability is often interpreted as noise to which conclusions should be robust. However, as I show in chapter 2, repeating experiments across a range of natural conditions can not only demonstrate the generality of findings, but also informatively account for differences in treatment outcomes between these repeats. In this case, apparent inconsistencies across years in the effect of chick treatment were quantitatively associated with the favourability of environmental conditions, and within a season, contrasting effects of both chick and parent treatment on different nests were quantitatively associated with hatch date. The mechanisms underlying these associations remain unclear, but the patterns nonetheless suggest that, in this system at least, we might better anticipate the role of parasitism in the outcome of a particular breeding if we account for the prevailing environmental conditions. Moreover, the impact of parasitism for individual family members under a given set of conditions may be better anticipated by considering certain aspects of the individual's phenotype, for example hatching order in chicks. In addition, the persistent effects of chick parasitism for parents described in chapter 5 illustrates that even in a year where we might expect a negligible direct impact of parasitism for certain family members, infection may still have implications for the performance of other family members.

Overall, this interplay between parasite impacts and environmental variability encourages a more constructive approach to the necessity of repeating experiments in wild populations across seasons. In chapter 2, the effect of treatment in the most productive year was the opposite to the less productive years, indicating that the effect of parasitism on an eco-evolutionary timescale could be either over- or underestimated by studies not covering a sufficient range of ecological conditions. The information we can gain from such natural variability can inform our understanding of the system, and may even be a step towards acknowledging more concretely the vast complexity of the eco-evolutionary questions that interest us.

In particular, my results suggest that the variability of parasite impacts with environmental conditions is not an intuitively simple case of marginal costs of parasitism decreasing steadily as conditions improve. Many previous studies have shown the importance of resource availability to how an individual copes with parasitism (e.g. Brzek & Konarzewski, 2007; O'Brien & Dawson, 2008; Pedersen & Greives, 2008; Ponton et al., 2011). However, although the cost of immunity is well established (Hasselquist & Nilsson, 2012) and it is intuitive that this should vary with environmental conditions, as it is intuitive that an animal in poorer condition is likely to suffer greater marginal costs of a given parasite infection, few parasite manipulation studies in wild populations take into account variability in prevailing environmental conditions in different years of experiments (Sandland & Minchella, 2003; Wolinska & King, 2009; Boughton et al., 2011). To my knowledge, chapter 2 of this thesis is the first quantification of how prevailing environmental conditions influence the impact of anti-parasite treatment on individual wild hosts, suggesting that the variability of parasite impacts between years reflects of a suite of ecological trade-offs. My environmental variable, productivity, integrates many ecological factors such as weather and food availability (Frederiksen et al., 2007b; Burthe et al., 2012). It would be interesting, though challenging, to investigate the separate effects of these different factors on the impact of parasitism, for example by feeding supplementation experiments to separate food availability from abiotic factors, or by further repeating similar parasite removal experiments across qualitatively different years such that, eventually, inter-annual (or indeed spatial) variation in parasite impacts could be modelled using the environmental variables themselves rather than an integrated proxy.

The general approach in parasite manipulation studies on life-history traits tends not to consider environmental variability in the interpretation of results unless it leads to unexpected outcomes of the manipulation, as in Knowles et al. (2010b). In that two-year study, anti-parasite treatment generally had a more beneficial effect for great tits' reproductive success in the less favourable year, as one might predict (Stearns, 1992; Sandland & Minchella, 2003). In a similar vein, experimentally reduced parental effort in Eurasian kestrels *Falco tinnunculus* decreased malarial infection intensity in mothers more in years of poorer food availability (Wiehn & Korpimäki, 1998). The general lack of consideration of environmental effects in these individual-level studies contrasts with many studies of the population-level effects of parasite manipulation, where the effects of parasitism are increasingly considered in concert with other drivers of population dynamics, such as population density or food availability (Hudson et al.,

1998, 2002; Albon et al., 2002; Vandegrift et al., 2008). My findings in chapter 2 are in keeping with the pattern of a greater benefit of treatment in less favourable conditions (for last-hatched chicks), but also add an interesting perspective in that in a very favourable year, the effect of treatment was not negligible, as one might predict, but negative. Although this could indicate a direct negative action of the drug, it is not clear why this should occur only in one year of the four. The result could also suggest a more complex trade-off between growth and dealing with an infection, incorporating other constraints such as parasite co-infections and sibling competition, rather than simply variability between seasons in marginal costs, which has previously been a sufficient explanation. In keeping with the caveats I raise above of a restricted range of study systems, these findings demonstrate the value of broad range of experimental conditions upon which to develop general ecological rules.

Similarly, within a season, variability in anti-parasite treatment effects may yield information on the influence of external conditions, and in some systems also intrinsic differences, of individuals' life history decisions in relation to parasite infection. In the shags, a marked influence of timing of breeding on parasite impacts is not unexpected as earlier breeders tend to be older, more experienced, more successful at rearing chicks and perhaps of better overall phenotypic quality (Harris et al., 1994; Daunt et al., 1999, 2006a, 2007b) and, moreover, host lower worm burdens (Burthe et al., 2013). Given these seasonal trends, we might expect that the costs of parasitism and thus the benefits of treatment would be greater for late breeders, as has indeed been previously found for adult shags (Reed et al., 2008). However, displaying another aspect of between-season variability, the effects I found of adult treatment do not fit the same pattern. Reed et al. (2008) found that adult treatment only increased survival of male chicks, and that only late in the season; in contrast, I found no sex differences in the survival effect, which occurred predominantly early in the season. Although these patterns might initially indicate that the conditions early in my study were similar to conditions late in Reed et al. (2008)'s study, my year was in fact more favourable than theirs, on which basis we we would superficially expect the opposite. As yet we do not have sufficient data to formally test these interannual differences in adult treatment effects, not least because the predicted interaction would be in four ways, between environmental conditions, treatment, chick sex and lay date. Thus far, a qualitative comparison does not suggest a clear pattern (fig. 6.1). The difference in treatment effect between years for parents might be more complex than for chicks if they are making decisions on current reproductive investment traded off against past or future

effort, whereas we expect chicks to aim predominantly to fledge in good condition. Overall, parasitism, prevailing conditions and timing of breeding all have the potential to affect the cost of current reproduction and thus influence this trade-off, which could lead to complex and even counterintuitive patterns.

How these patterns scale up to affect populations, or individuals in the longer term, may interplay closely with within- and between-season variability in parents' breeding success and chick recruitment. Earlier breeders are generally more experienced and more successful (Daunt et al., 1999), and in addition chicks hatched early in the season are more likely to recruit (Harris et al., 1994). Late breeders may thus contribute little to the breeding population, suggesting that if parasite infection has any demographic importance for shags, the effect is likely to be driven by its impact on earlier breeders. Hence, the subtle but apparently negative of effect of anti-nematode treatment I found for late breeders in 2011 may be negligible at the population level; by extension, the evolution of the host-parasite relationship could perhaps be driven predominantly by those early families' responses to infection. Between seasons, variability in recruitment is more stochastic as it is strongly affected by winter weather, as with adult survival (Harris et al., 1994; Frederiksen et al., 2008), but a similar argument applies: how family members deal with parasite infection is likely to be of greater consequence to population processes in years producing more recruits and returning breeders.

6.2.1 Variability in parasite exposure

A major consideration for these patterns of temporal variation in parasite impacts on hosts is how worm prevalence may vary through time, both within and between years. This remains a substantial unanswered question for this study system. The only pattern known with certainty, from a single-year study (in 2011) of *in situ* parasite burden of adult shags, is that worm burden was highest for late-nesting males and lowest for early-nesting females (Burthe et al., 2013). However, it is not yet clear whether later birds have higher worm burdens as a correlate of being of poorer quality overall, which could entail poorer immunocompetence, or whether the abundance of infective larval stages increases through the season. The latter possibility is plausible at least: the worms' life cycle is probably completed within a matter of weeks (see appendix A), with eggs shed in the shags' faeces. As the breeding season progresses over several months, worms could therefore become more abundant in the relatively restricted feeding areas that the shags use in the summer compared to over the winter. Indeed,

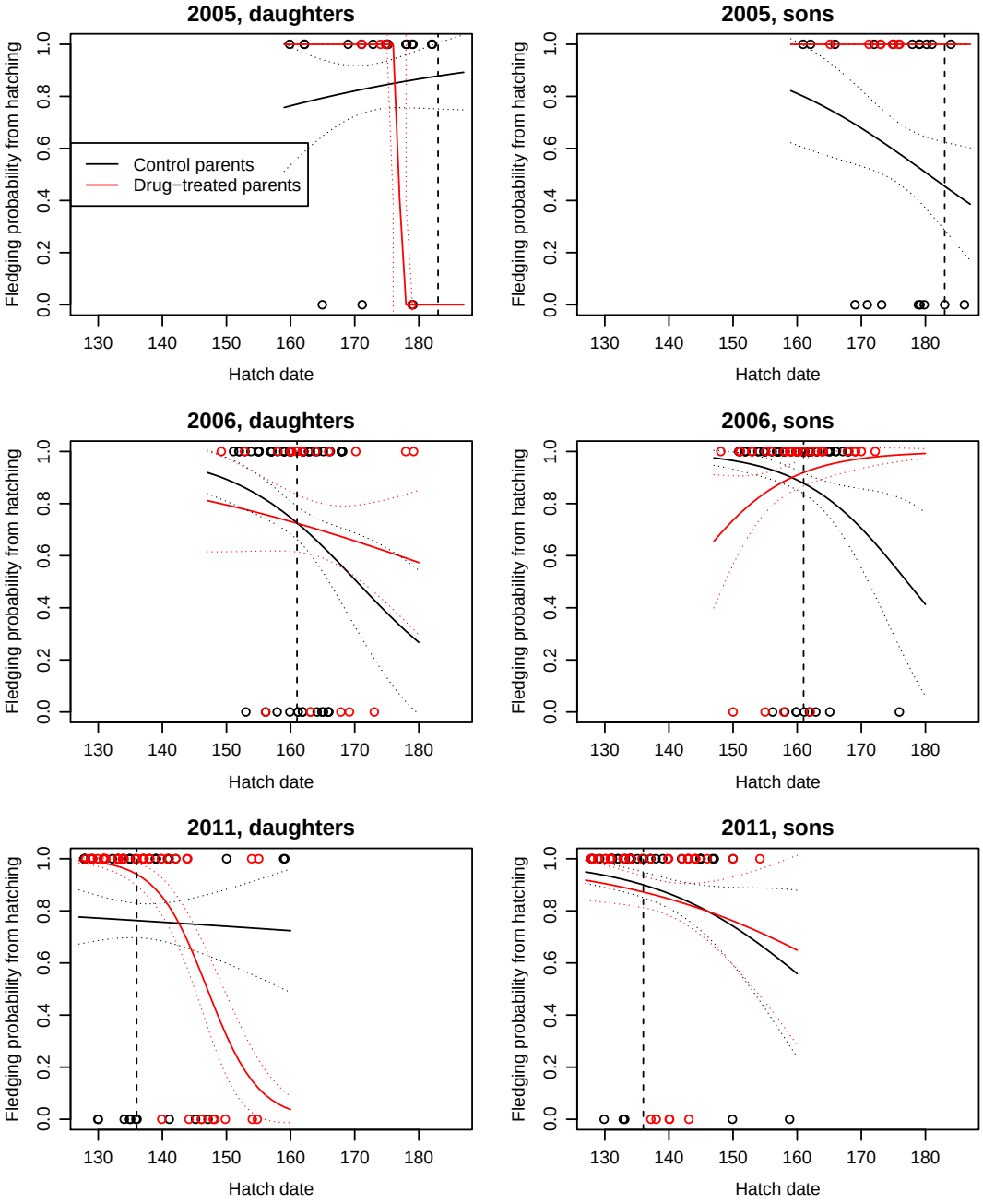


Figure 6.1: The between- and within-year variability in the effect of adult treatment on survival of daughters (left panels) and sons (right panels). Plots show the patterns from all three years of adult treatment studies to date (2005 and 2006 from Reed et al. (2008), 2011 from chapter 3). The phenological pattern is shown against absolute hatch date, but with the median hatch date each year marked by a dotted line. The environmental conditions were worst in 2005 and best in 2011, with average fledged brood sizes of: 0.48 in 2005, 1.22 in 2006 and 1.54 in 2011.

worm prevalence in pellets of undigestable material produced by shags has suggested a seasonal increase in exposure to some nematodes (Reed et al., 2008). Importantly, however, there is much less known of seasonal or annual patterns of worm prevalence in chicks than in adults (but see appendix A).

There may also be variation between years in worm prevalence or exposure, for example if the abundance or distribution of their intermediate hosts differs with environmental conditions (Frederiksen et al., 2007a) or if shags' summer feeding areas vary between years (M. Bogdanova, pers. comm.). Such factors could not only affect overall parasite impacts but perhaps even lead to different seasonal patterns in different years. This could be of substantial ecological importance if previous parasite exposure has additive consequences, as in Alpine swifts *Apus melba*: a three-year study showed that the effect of chick ectoparasitism on parents' reproductive success was cumulative, with fleas in the nest reducing parents' subsequent breeding success more if parents had reared parasitized broods the previous year (Bize et al., 2004). If the same is true of shags, repeated use of the same feeding areas from year to year could cause cumulative costs for parents.

6.3 Persistence of parasite effects

As well as effects of anti-nematode treatment not being confined to individuals, I found that they were not confined to the breeding season (chapter 5). Parents' subsequent breeding timing was influenced by chick treatment but not by their own treatment, reflecting the physiological impact of treatment described in chapter 3. This finding supports the indication from the physiological and behavioural impacts of treatment discussed above that chick parasitism may be of greater consequence to resource distribution among the family, and hence parents' fitness, than parents' own parasite burden. The persistence of this impact is a further indication that current paradigms may not fully appreciate the fitness consequences of parasitism in families, as examining parasitism within a single breeding attempt may overlook important longer-term impacts of infection.

Earlier breeding, associated with greater fledging success and more recruits produced (chapter 5; Harris et al., 1994; Daunt et al., 1999; Reed et al., 2008), was linked to lower foraging effort in the preceding winter, indicating a potential association between winter foraging on productivity and hence parental fitness. Winter foraging did not have direct fitness consequences in itself in the year of this study, in which

non-breeding conditions were not severe (Newell et al., 2012). However, under less favourable winter conditions, lower foraging requirements could have more substantial impacts on condition, and perhaps even mortality, as shags are vulnerable to starvation under adverse winter weather (Daunt et al., 2006a; Frederiksen et al., 2008). In this relatively long-lived species, with three years to sexual maturity and subsequently many breeding attempts per individual (often over 10 breeding seasons in a lifetime), adult survival may be a particularly sensitive demographic variable. Poor conditions during the winter may also have a less extreme effect by causing shags to skip breeding in the subsequent year, which may play as great a role as mortality in causing very low productivity in "crash" years (Aebischer & Wanless, 1992; Frederiksen et al., 2008). Thus, it is imaginable that a year of high chick parasite prevalence followed by a severe winter could reduce the breeding population in the following year.

Further, the effect I found of chick treatment on parents' subsequent breeding (chapter 5) suggests that parents' responses to parasitism during chick-rearing may not be purely passive, i.e. only reacting to minimize the immediate costs of infection. Irrespective of differences across the season in the immediate effects of treatment, longer-term effects were the same for all drug-treated birds, both early and late, suggesting that there may be an adaptive component to shag parents' responses to parasitism in their chicks. This pattern could, however, also be explained by immediate and longer-term effects arising through different physiological mechanisms, the former varying with hatch date and the latter not. In either case, this result contrasts with findings from another long-lived species, the Alpine swift, where chick parasitism had negative effects on parents both immediately and in subsequent breeding (Bize et al., 2004). That study, however, acknowledged that manipulation of chicks' parasite burdens had a correlated impact on parents' parasite burden, so the effects cannot confidently be attributed to chick parasitism alone. All in all, my demonstration that chick parasitism can influence parental fitness traits continuously for at least a year indicates a potentially substantial role for offspring parasitism in parents' trade-offs between current and future reproduction.

6.4 Implications for populations

The work in this thesis offers insights into the potential for parasites to influence seabird populations. The impact of adult treatment on chick survival and chick treatment on chick growth, both key demographic rates, could conceivably have implica-

tions for population processes. However, some care should be taken taken in interpreting the ecological significance of these measures.

I have generally interpreted increased growth rate as a positive effect (discussions of chapters 2 and 3) as fledging mass is associated with survival in a range of bird species (Magrath, 1991; Schwagmeyer & Mock, 2008). If this is a causal link, such that greater fledging mass leads to increased survival, then this interpretation holds. However, the link may also be a correlational reflection of overall phenotypic quality, with better individuals both growing faster and surviving better. In that case, parasitism could alter growth without affecting survival, and this would explain previous findings of chick parasitism reducing fledging mass but not affecting recruitment (Heeb et al., 1999; Fitze et al., 2004b). Nonetheless, in shags, structural growth rate has been shown to be remarkably robust to food deprivation, with larger effects on the size of internal organs (Moe et al., 2004a). It is thus reasonable to assume that slower growth would be detrimental, as smaller organs would be a disadvantage to this highly aerobic predator. One of my dissections is particularly illustrative here. An almost-fledged chick that had died naturally, presumably through starvation or disease, had a wing length in keeping with its age but also very small organs that left empty space in the body cavity and, moreover, carried a huge worm burden (details in appendix A). In particular, the size of the heart and lungs was typical of a chick less than half its age, with clear implications for its underwater foraging ability. Another consideration in interpreting growth rate is that growth after the linear phase may counteract changes to earlier growth, such that linear growth rate does not reflect fledging mass (but see Reed et al. (2012)), and moreover, fledging size in itself may not be the only determinant of future success. Perturbation to growth trajectories can be detrimental, with delayed development followed by accelerated "catch-up" growth in particular shown to have long-term effects on a variety of fitness traits (reviewed in Metcalfe & Monaghan, 2001; Mangel & Munch, 2005; Monaghan, 2008). To examine whether parasitism produces such effects in shag chicks would require following chicks beyond the linear growth phase (10–30 days, Daunt et al. (2001b)) until treatment has worn off after at least 18 days (Burthe et al., 2013) (i.e. age 28 days following treatment at 10 days), both of which coincide with chicks becoming more mobile. I did not pursue these questions for this thesis due to the risk of premature fledging, but careful selection of low-risk nests (away from steep cliffs) could allow a more detailed investigation of parasite impacts on growth trajectories.

The effect of anti-nematode treatment on chick survival should also be interpreted with some caution. If a last-hatched shag chick does indeed serve a resource-tracking function, theory predicts that its death should benefit the remaining chicks by increasing their share of resources (Temme & Charnov, 1987; Mock & Forbes, 1995; Stenning, 1996). Thus, while the death of a chick is clearly detrimental to its individual fitness, the dead chick and its parents may gain inclusive fitness from the increased likelihood of its siblings recruiting. Indeed, Heeb et al. (1999) found in great tits that although ectoparasitism of chicks decreased fledging success, recruitment was not affected. This question could feasibly be addressed by monitoring the recruiment of experimental chicks once sexually mature at the age of 2 or 3 years (Wanless & Harris, 1997). Such long-term monitoring could also inform the interpretation of growth rates.

My findings illustrate a further aspect of complexity in how individual-level parasite impacts might be reflected at the population level, namely that different individuals may suffer different impacts. This suggests that the ultimate impact of parasites on population processes may depend on the composition of the population. In chapter 2, I showed that last-hatched siblings in a brood are more strongly affected by anti-nematode treatment, and furthermore that this within-brood difference was greater in less favourable environmental conditions. Thus, in a less productive year, last-hatched chicks are particularly vulnerable to parasitism. However, last-hatched chicks also had higher mortality in poorer years, such that the population-level impacts of the within-brood difference may not differ considerably between years if in poor years there are fewer C chicks to be heavily impacted by parasites. Previous studies have also found the effect of parent parasitism to be more detrimental to sons than to daughters, and more beneficial to mothers' foraging than to fathers. However, in general in this thesis I have found no substantial sex differences among either chicks or parents, in contrast to what we might expect given the shags' sexual dimorphism. The sex difference may be small enough that it is only noticeable in less favourable years than ours, which were all above average in terms of productivity (Newell et al., 2012).

These caveats notwithstanding, the treatment effects that I have demonstrated on individuals have the potential to scale up to influence population processes. This scaling up may depend not only on the longer-term impacts of the demographic rates we measured, but also on how parasites are transmitted between hosts. In particular, it is possible population-level foraging characteristics might influence the transmission of these water-borne, trophically transmitted nematodes. If transmission is low, impacts are likely to be more confined to individuals and the population-level impact may be

relatively small. However, it is conceivable that factors such as high foraging density or consistency in foraging areas could increase transmission by concentrating definitive hosts, and hence perhaps also egg shedding, in a restricted area. For example, shags share foraging areas on sandy sea floor with other conspecifics while foraging density is lower in rocky areas, presumably because sandy areas contain more profitable prey (Watanuki et al., 2008). A greater prevalence of infective worm larvae might thus build up in such areas, even though indivduals forage across both habitat types (Watanuki et al., 2008). Characteristics of the intermediate host populations, such as dispersal of fish, are also likely to influence parasite transmission. In addition, the principal nematode in the system, *Contracaecum rudolphii*, is not specific to the shag but will infect any seabird host (Hoberg, 2005; Fagerholm & Overstreet, 2008), so its transmission may also depend on the susceptibility and behaviour of these other species. On the other hand, the shag's foraging niche does not substantially overlap with other seabirds along the Scottish coast (which are either surface feeders or deeper pelagic divers, not inshore benthic divers like the shag), so are unlikely to be exposed to the same parasite populations. Without more detailed knowledge of the movement patterns of the intermediate hosts, and indeed more detailed knowledge of what species these primarily are, we cannot speculate on the role that other seabirds might play in the transmission of worms to shags.

6.5 Benefit of anti-parasite treatment

In interpreting the findings of this thesis and the role of nematode infection, it is worth noting that the anti-parasite drug that I used (ivermectin) may also affect other macroparasites, including ectoparasitic arthropods such as the biting lice that shags are also infected with and are particularly prominent on chicks (Daunt et al., 2001a; pers. obs.). However, a previous study carried out in a year of below-average productivity found no association between louse burdens and breeding success of experimentally delayed or advanced nests (Daunt et al., 2001a). Moreover, recent work using ivermectin in rodents suggests that its action on arthropods is negligible (Pedersen & Antonovics, 2013; Knowles et al., 2013). In addition, from my dissections it appears that gastrointestinal worms overwhelm ectoparasites in terms of their sheer volume, and ivermectin has specifically been shown to be effective against nematode in shags (chapter 2; appendix A; Burthe et al., 2013). Therefore, the action of the drug on nematodes is likely to have contributed substantially to the observed effects of anti-parasite treatment for the shags, although this could have been via knock-on effects on other

gastrointestinal parasites of altering the parasite community (Pedersen & Antonovics, 2013; Knowles et al., 2013) rather than a direct consequence of reducing the numbers or decreasing the activity of the nematodes. Nonetheless, this thesis thus provides a valuable addition to our understanding of parasitism in indivudal host ecology and life-history, particularly among birds, examining the impacts of endoparasites rather than ectoparasites or microparasites. Endoparasites are logistically more difficult to quantify and reliably manipulate and have thus been relatively poorly studied in the context of families.

We must also consider the assumption running through this thesis that nematode infection is entirely detrimental to the host. While this is probably true in terms of resource costs – requiring an immune response, damaging tissues and directly withdrawing resources from the host's gastrointestinal tract – nematodes could interact with other infectious agents, potentially leading to counterintuitive outcomes. In mammals, nematodes have been shown to suppress certain components of the host's immune response, facilitating infection by other parasites and pathogens, which could increase their negative effect (Ezenwa & Jolles, 2011). However, nematodes may also compete with other infections for resources within the host (Graham, 2008), preventing the establishment of other, potentially more pathogenic parasites. This may be particularly true for parasites sharing the same gastrointestinal niche as the worms, such as intestinal protozoans (Knowles et al., 2013). Indeed, ivermectin has recently been shown in mice to cause an increase in gastrointestinal coccidian and cestode prevalence, most likely as a consequence of altered co-infection dynamics when the nematode burden is reduced (Pedersen & Antonovics, 2013). Moreover, recent pilot data suggest a negative correlation between coccidia and nematode infections in adult shags (J. Benton & F. Walker, unpubl. data). To date, little else is known of co-infection dynamics in the shag between nematodes and other parasites – microparasites, other helminths or ectoparasites – but this is a promising avenue for future work.

6.6 Future work

This study has opened interesting questions about the role of parasites in host reproduction and life-history, including more specific questions regarding many aspects of the shag/nematode system, which I have outlined above. Here, I summarize the three questions which I feel would be most rewarding, most informative to my questions in particular, and most feasible.

The most fundamental, but also most difficult to address, is the question of how shags' parasite exposure varies between individuals, within seasons and between years. As yet it is not known, for example, whether shags face a constant infection rate throughout the breeding season, whether winter foraging areas have lower parasite prevalences, or whether variability between individuals outweighs extrinsic sources of variation. Recent data suggest that individuals may be repeatable in their burdens from one year to the next (S. Burthe, unpubl. data), which also raises the question of whether individual variation is driven by exposure, for example through foraging areas, or by susceptibility to infection. The shags' main prey species, the lesser sandeel *Ammodytes marinus*, is presumably the main paratenic host for the nematodes to infect the shags, but these fish are not easy to sample in the relevant foraging areas, and surveying them for encysted larvae would be time-consuming. Given, therefore, that direct measurement of parasite distributions is very challenging, I hope that this sort of information will build up by other means as the study continues.

The second question, most informative to the role of parasites in family conflict, would be that of how siblings might vary intrinsically in their ability to deal with infection. I showed that hatching order was a key determinant of chicks' responses to parasitism across a range of environmental conditions, but could not identify a single mechanism to explain this. To my knowledge, no study has previously examined maternal antibody concentrations in clutches of the Phalacrocoracidae (cormorants and shags). Examination of young shags' antibody levels, and the devlopment of immune responses with time, could illuminate this observation.

Finally, a feasible and ecologically important avenue for continued research on this system should be to follow the longer-term consequences of the treatments that have been carried out to date. The vast majority of parents and all chicks that have so far been involved in parasite manipulations in this population (Reed et al., 2008, 2012; this thesis) are ringed and thus individually identifiable. Shags are philopatric, with 90% of experimental chicks that survive to breeding age expected to recruit to the Isle of May, and site-faithful once recruited, with $>$99% of experimental adults expected to breed on the Isle of May until death (Barlow et al., 2013). All breeding attempts at the colony are fully surveyed every year, and in addition there is currently substantial monitoring effort of the more dispersed winter distribution of shags along the Scottish east coast, increasing the accuracy of survival estimates. It is thus not inconceivable to investigate how both chick and parent treatment affect parents' future reproduction and chicks' recruitment, even for the rest of the lifetimes of the experimental birds.

6.7 Conclusion

This thesis has demonstrated the importance of parasitism during reproduction for the fitness of all family members, and illustrated complexities in the interpretation of these effects depending on intrinsic differences between individuals, environmental conditions and their interactions with other family members. With this, I suggest that the role of parasitism is generally underappreciated in our understanding of hosts' reproductive decisions and life-history trade-offs and in offspring development. A similar underestimation of the role of parasites has been suggested in community ecology: food web theory has almost entirely overlooked parasites, and incorporation of parasites into traditional theory is difficult given the frameworks that exist (Sukhdeo, 2010). In contrast, the inclusion of parasitism would not require adjusting our current models of resource-mediated life-history trade-offs. Rather, parasitism needs to be considered more often in the interpretation of reproductive decisions. In concert with this consideration, evolutionary ecological research would gain from a better understanding of the parasite communities facing the study species in all contexts; such an understanding is currently confined to studies specifically addressing the role of parasites in host ecology. This thesis suggests that by embracing deviations from well-established theory, we might build a more holistic, complex but accurate understanding of the ecological interactions that we find so fascinating.

APPENDIX A

Basic parasitology in the shag-nematode system

A.1 Introduction

In this thesis, I have investigated various facets of the costs of parasitism during the breeding season, using the host-parasite system of European shags *Phalacrocorax aristotelis* and their gastrointestinal nematode parasites, predominantly *Contracaecum rudolphii*. Several studies on the shag/nematode system have demonstrated by experimental parasite removal that nematode infection influences the physiology and behaviour of both parents and chicks (Reed et al., 2008, 2012; this thesis). However, many aspects of the biology of the parasite remain unknown, and my findings have highlighted several areas where more information would be desirable. Quantitative measures of the extent of infection are also rudimentary, although recent pioneering work on adult shags has demonstrated that endoscopy of conscious birds is a reliable method by which to quantify worm burden. Moreover, this thesis and previous work have found differences between individuals in how they are affected by anti-parasite treatment according to factors such as sex, hatch date and, for chicks, position in the brood hierarchy. This could be because individuals carry different parasite loads or because they differ in their ability to deal with the costs associated with a particular

burden. Accurate quantification of individual shags' parasite burdens would be a major step forward in untangling the relative importance of these effects.

In this appendix, we describe and attempt to address both the knowledge gap in the natural history of the shag-*Contracaecum* system and the problems of quantifying parasite burdens across individuals. We review the available literature to describe the life history of the nematodes and to assess their species identification (section A.2). We then present data on three methods of parasite quantification: endoscopy, faecal egg counts and dissections (section A.3). We have extended the use of the novel technique of endoscopy to chicks, and compare this to dissections of opportunistically recovered naturally dead chicks, the only other available method of *in situ* parasite quantification. Further, we assess the reliability of faecal egg counts as an indicator of infection in this system by comparing both *in situ* measures of worm burden to faecal samples taken from the same chicks.

A.2 Literature review

Gastrointestinal nematodes are almost universal parasites of wild vertebrates (Atkinson et al., 2009). However, nematodes of seabirds receive relatively little attention as most such systems are not of direct medical or financial importance (Fagerholm & Overstreet, 2008). Nonetheless, seabirds play a key role as apex predators in many marine ecosystems, and as such also offer a tractable indicator of the state of less visible parts of the ecosystem (see introduction to this thesis, section 1.1). If we wish to incorporate the role of parasitism into our understanding of seabird population dynamics, we require knowledge of the biology of the parasites themselves. Like most wild animals, seabirds can host a huge range of parasites: protozoan blood parasites, intestinal protozoans such as coccidia, several groups of worms (helminths) and ectoparasitic arthropods such as lice and mites (Atkinson et al., 2009). Among helminths, seabirds have been reported to host flatworms, cestodes and acanthocephalans as well as nematodes (roundworms) (Hoberg, 2005). In this thesis, we focus on gastrointestinal nematodes. Nematodes are a prominent and characteristic component of birds' parasite fauna (Hoberg, 2005), making them both relatively easy and important to study. *Contracaecum* infections in particular may consist of hundreds of individual worms (Hoberg, 2005), indicating that they may pose a considerable cost on their host, at least in terms of competition for resources.

A.2.1 Nematodes of shags

To date, three species of nematode have been identified in the shag in Western Europe: *Contracaecum rudolphii* in Scotland and Spain (Abollo et al., 2001; Reed et al., 2008), *Contracaecum septentrionale* in Spain (Abollo et al., 2001) and *Anisakis simplex* in Scotland (Reed et al., 2008). In addition, one study reports a species of acanthocephalan (*Andracantha tunitae*) in shags from northern Scotland with 69% prevalence (Munro et al., 1995), and another study reports a substantial infection of an acuarioidean nematode in the gizzard of a single chick but gives no further details (Snow, 1960). Of the nematodes, *C. rudolphii* and *A. simplex* have been identified specifically in our study population.

A. simplex was identified from shags at our study site, the Isle of May, in 2005, dissected from dead chicks and extracted from regurgitated pellets of undigestable material from adults. In both cases, the *A. simplex* were identified as third- or fourth-stage larvae (L3 or L4; H.-P. Fagerholm & T. Reed, pers. comm.). The definitive hosts of *A. simplex* are marine mammals (cetaceans and pinnipeds) (Anderson, 2000; McClelland, 2005) and we are not aware of any certain record of adult *A. simplex* in an avian host. However, because *A. simplex* is common in many fish species (an intermediate host) (Anderson, 2000), it is not surprising that L3 *A. simplex* should be found in shags' stomachs. The moult to L4 may even be successful in a foreign host, but to find an adult *A. simplex* in a bird would be highly unusual (H.-P. Fagerholm, pers. comm.), so while L3 *A. simplex* may be relatively common it is unlikely to reach the reproductive stage in shags. *C. rudolphii*, on the other hand, is a bird parasite, as are many other species of *Contracaecum* (Anderson, 2000; Fagerholm & Overstreet, 2008; Moravec, 2009).

Contracaecum infections are very common in piscivorous birds, and *C. rudolphii* is considered the most common species, partly because of its broad geographical and taxonomic range (Anderson, 2000; Fagerholm & Overstreet, 2008). All adult worms that have been identified from our study population, from both chicks and adults, have been *C. rudolphii* (S. Burthe & E. Harris, pers. comm.). A study on a Spanish population of shags found *C. rudolphii* in all individuals examined. Infection intensities of *C. rudolphii* were many times greater than a congeneric nematode, *C. septentrionale*, which was found in only 15% of shags examined (Abollo et al., 2001). Fagerholm & Overstreet (2008) report that of all cases of *C. rudolphii* infection in the literature, 32% were from *Phalacrocorax* species. In addition, Huizinga (1971) re-

ports *C. spiculigerum*, which several authors suggest is synonymous with *C. rudolphii*, (Fagerholm & Overstreet, 2008; Moravec, 2009) from 96% (45 out of 47) of dissected double-crested cormorant individuals and all of 11 dissected European cormorants. *C. rudolphii* (Hartwich 1964) itself is now recognized to consist of a complex of morphologically similar sibling species which have yet to be formally identified (Fagerholm & Overstreet, 2008; Moravec, 2009). Throughout this study we use *C. rudolphii* in the broad sense to include the whole species complex, and include studies of *C. spiculigerum*.

A.2.2 Costs of nematode infection in seabirds

Uncertainties of identification notwithstanding, both candidate species (*C. rudolphii* and *A. simplex*) are members of the same subfamily of nematodes, the Anisakidae (within superfamily Ascaridoidea), with similar life histories (Anderson, 2000; McClelland, 2005). Their effects on seabird hosts have previously been assumed to be subtle, so nematode infection has traditionally been considered of little consequence to the bird's ecology or even "benign" (reviewed in McClelland, 2005). Nonetheless, nematode infections are often reported to be associated with sub-lethal effects such as emaciation and lethargy in seabirds, and occasionally even mortality, although a causal relationship is rarely evident (Galaktionov, 1996; McClelland, 2005; Hoberg, 2005; Fagerholm & Overstreet, 2008). In several species of marine and shore birds, high parasite diversity or infection intensity has been associated with breeding failure, though again the causality is unknown (Hoberg, 2005). Nematode infection probably has the most noticeable negative impacts in combination with unfavourable environmental conditions, for example exacerbating the effects of starvation (Galaktionov, 1996; Hoberg, 2005; Fagerholm & Overstreet, 2008).

Cross-sectional studies suggest that standard physiological indicators in seabirds such as body condition or fat score are commonly not affected by gastrointestinal nematodes (Hoberg, 2005; Fagerholm & Overstreet, 2008). Instead, their effects are likely to be subtle but potentially long-term: for example, in the Laysan albatross *Phoebastria immutabilis*, nematode infection is associated with delayed moult, which could delay breeding, reducing success, or even cause the bird to skip breeding entirely (Langston & Hillgarth, 1995). However, despite several reports of negative associations between nematode infection and host fitness measures in seabirds, the impact of many marine nematodes on their hosts remains poorly studied, particularly at the pop-

ulation level, let alone their role in host ecology. Because associations with illness or mortality are usually observed as singular, chance events, it is difficult to build a general picture of nematodes' impact on populations (Galaktionov, 1996; Hoberg, 2005). Given the natural variation in prevalence and sub-lethal effects usually associated with gastrointestinal nematodes (Clayton & Moore, 1997), this would require longitudinal studies of individual hosts, but non-destructive quantification of these parasites is difficult. This challenge is the subject of section A.3 below.

The pathology caused by anisakids is generally confined to the gastrointestinal tract, arising where worms attach to the host's stomach wall (McClelland, 2005). Both larvae and adults may cause lesions, ulceration, tissue necrosis, haemhorraging, perforation of the stomach wall, inflammation and secondary bacterial infections (Kuiken et al., 1999; Hoberg, 2005). In shags, lesions at attachment points have been frequently noted during endoscopic examination of adults (S. Burthe, pers. comm.). Fagerholm & Overstreet (2008) observe that inflammation may be particularly severe when juvenile worms embed in the stomach wall, indicating that L3 may be the worm's most pathogenic life stage. This could be important in determining the relative costs of *A. simplex* and *C. rudolphii* for the shags: *A. simplex*, a parasite of mammals, may have a substantial impact on shags if it is able to attach, which as yet remains unknown. Such tissue damage may only be transient, as indicated by Huizinga (1971) who, in dissected double-crested cormorants, found lesions from worm attachments in only 9 of 45 infected birds. However, transient damage may lead to persistent costs of tissue repair and, moreover, this immediate pathology may be compounded by secondary bacterial infections, potentially increasing the chance of death (Fagerholm & Overstreet, 2008). Chicks may suffer more severe pathology, with fibrosis more common at attachment points and pathology extending deeper through the stomach tissue layers than in adults (Fagerholm & Overstreet, 2008). How the worm burden might change with age is not clear, however: in pelicans, nematode infection intensity decreased with age (Humphrey et al., 1978), but in double-crested cormorants, prevalence increased to 100% among post-fledging chicks (Kuiken et al., 1999).

Worms may impose further costs on their hosts beyond immediate pathology. Birds are likely to mount an immune response against the worms, which can be costly (Colditz, 2008; Hasselquist & Nilsson, 2012). Worms may also modulate the immune response in more complex ways, and thus affect the host's ability to deal with other infections. An immunosuppressive effect of *A. simplex* has been demonstrated *in vitro*, with its excretory products inhibiting the proliferation of rodent lymphocytes (Ray-

bourne et al., 1983), and we might predict a similar effect of *A. simplex* or even *C. rudolphii* in birds. Nematodes may also be costly because of their sheer volume: a heavy infection may reduce the space available for food in the host's digestive tract, with implications for seabirds in particular in terms of foraging efficiency, feeding trips length and chick provisioning (Hoberg, 2005). In addition, anisakids in general and *Contracaecum* in particular appear to feed on fish ingested by the bird, rather than feeding on the host itself (Dubinin, 1949; Huizinga, 1971; Anderson, 2000; Abollo et al., 2001), putting them in direct competition with the host for resources. On the other hand, this may lead to a beneficial effect of nematode infection: several authors have suggested that the worms' feeding movements could help break up the ingested food, making digestion more efficient (Dubinin, 1949; Huizinga, 1971; Fagerholm & Overstreet, 2008).

A.2.3 Life cycle

The lifecycle of *C. rudolphii* has not been well studied in comparison to medically or economically important parasite species (Fagerholm & Overstreet, 2008), although compared to other marine nematodes, the anisakid life cycle is relatively well known (Anderson, 2000). *A. simplex* and *Pseudoterranova decipiens*, for example, are known to release eggs in the faeces of their hosts (cetaceans and pinnipeds); larvae then pass through invertebrate hosts into fish, which transmit the larvae to the definitive host (McClelland, 2005). For *C. rudolphii*, Fagerholm & Overstreet (2008) present a synthesis of information from closely related species that builds a relatively complete – though not certain – picture of this worm's transmission and reproduction. Moreover, three isolated experiments on the reproductive biology of *C. rudolphii* provide some detail on timing of each life stage (Dubinin, 1949; Bartlett, 1996; Moravec, 2009).

The following overview is expanded from Fagerholm & Overstreet (2008). In seabirds, the definitive host, adult *C. rudolphii* produce eggs that are released, fully embryonated, in the bird's faeces. Two moults happen within the egg, and the worms hatch as third-stage larvae (L3) into the water (Anderson, 2000; Moravec, 2009). A laboratory study found this to take 9–17 days in water at 15–20$^{c}ircC, which is substantially warmer and perhaps, therefore, faster than the North Sea, the setting for our study system. These are eaten by invertebrates, predominantly copepods, where they migrate to the body cavity and grow in the haemocoel (Anderson, 2000; Fagerholm & Overstreet, 2008). Worm maturation in the invertebrate host probably only lasts a

few days: an experimental study successfully infected fish by feeding them copepods exposed to worm larvae 7–12 days previously (Moravec, 2009). When the invertebrate host is eaten by a fish, the L3 worms encyst in the organs, such as the intestinal wall or liver, or mesentery, but not in the muscle tissue (Bartlett, 1996; Anderson, 2000; Fagerholm & Overstreet, 2008; Moravec, 2009). Larvae grow in the fish, reaching infectivity after 44 days according to one study (Bartlett, 1996), and may survive as larvae in the fish for over a year (Moravec, 2009). Both the invertebrate and fish hosts are paratenic, facilitating the progression of the worm to its definitive host, but have been shown in laboratory studies not to be necessary for the worm's life cycle (Dubinin, 1949; Moravec, 2009). These authors stress, however, that although *C. rudolphii* may not be obligately heteroxenous (life cycle through more than one host) physiologically, it does in ecological reality require both copepod and fish stages to infect seabirds.

When the fish is eaten by a bird, the worm establishes itself in the bird's proventriculus where it moults to L4 and finally to its reproductive adult form (Fagerholm & Overstreet, 2008; Moravec, 2009). Some evidence suggests that worms may live as adults for only about 3 months or less: two captive double-crested cormorant chicks spontaneously stopped passing *C. spiculigerum* eggs 14 weeks after capture (Huizinga, 1971), and an adult European cormorant dissected 80 days after experimental infection with *C. spiculigerum* contained no worms (Dubinin, 1949). In that case, ingestion of infected fish must be continuous in order for an infection to persist (Dubinin, 1949; Huizinga, 1971; Anderson, 2000; Fagerholm & Overstreet, 2008).

We know nothing of the natural range of intermediate host species of *C. rudolphii*, but copepods are probably important at the first stage and thereafter a broad range of fish species is likely (Anderson, 2000). In an experimental infection study, Moravec (2009) cites several studies supporting the function of copepods as intermediate hosts and, moreover, found low survival of other arthropod groups when infected. Furthermore, in the shag system, copepods are of huge ecological importance, underlying much of the trophic structure of the North Sea and acting as the main food source for the shags' main prey, the lesser sandeel, *Ammodytes marinus* (Frederiksen et al., 2007a). From both a physiological and ecological perspective on the transmission cycle in the shag-*Contracaecum* system, copepods are thus likely the most important first-stage paratenic hosts. At the second transmission stage, Moravec (2009) successfully infected a broad range of fish species with *C. rudolphii* larvae, which survived for almost two years in the fish. The fish infected included minnow, pike, guppy and loach, but only fresh-water species were tested, none of which our shag hosts would

feed on. However, it indicates the flexibility in host use for *C. rudolphii* at this developmental stage. In addition, Huizinga (1967) states that larvae of a congeneric worm, *C. multipapillatum*, are found in a variety of marine fish species. Our understanding of the role of nematodes in shags, in particular the temporal variability in their impact, would greatly benefit from further research into the range of intermediate host species and their distribution.

A slightly unorthodox study from the USSR yields interesting information on the timing of development once in the bird. (Dubinin, 1949) conducted experimental infections with the synonymous *C. spiculigerum*. The experiment uses cormorants, herons and pelicans, and is occasionally unclear which host species certain tests or numbers refer to. All birds were dewormed using a combination of copper sulphate, plant extracts and ricin oil over three days, which was confirmed by dissection to remove or decrease worm burdens in herons. The birds were then infected with larvae extracted from fish, either with encysted larvae in food (cooked fish) or by direct placement into the stomach, and dissected after an apparently arbitrary number of days. When worms were fed to the birds, over 50% of larvae (5 out of 8) had become established in the cormorant after 10 days, although it is not clear whether they were yet sexually mature. The experiment placing the larvae directly in the stomach suggested that maturation times were variable, with 24% of introduced larvae having reached adulthood after 10 days and 34% after 20 days (10 and 23 out of 68, respectively); note that it is unclear whether the birds are cormorants or pelicans. In a third experiment, three cormorants were fed larvae and dissected after longer periods to investigate the life span of the adult worms. Worm burden was low at 30 days after infection (6 adults from 30 larvae), and after 80 days, the host had no worms. In herons, the congeneric *C. microcephalum* reached sexual maturity in 6 days after larval introduction and infection persisted for at least 30 days. Of 248 larvae ingested with food, 129 (52%) were found established as adults after 20–30 days.

Chicks are infected predominantly with larval stages through food from the parents, and substantial infections may become established within a few days of hatching (Hoberg, 2005). Huizinga (1971) and Fagerholm & Overstreet (2008) suggest, without providing evidence, that direct infection with adult worms dislodged from the parent's proventriculus during feeding may be an important transmission route, as adult worms are found in food boluses in adult birds' stomachs that may be fed to chicks. Dubinin (1949) tested this possibility by infecting birds with both juvenile and adult worms dissected from other hosts, stained to ensure identification, and dissecting the recipient

hosts 5 days after the cross-infection. All worms established in the recipient host, even when this was a different species from the donor host, suggesting that direct transmission from parents to chicks is a theoretical possibility. Dubinin (1949) was motivated by the observation that large, sexually mature worms were sometimes found in one- to two-day-old cormorant chicks (which we did not find in the shags; see below). Huizinga (1971) describes three-week old cormorant chicks passing *C. spiculigerum* eggs in their faeces, i.e. hosting adult worms.

A.3 Quantifying parasite burden

A.3.1 Introduction

Quantifying endoparasite burdens is notoriously difficult. In wild birds, the only readily available *in situ* measure of worm infections is necropsy. The requirement of killing study animals makes this approach ethically complex and in certain populations, for example of protected species or those involved in long-term studies, it is not logistically possible. Moreover, killing the host restricts studies to be cross-sectional as it rules out longitudinal studies of individuals, for example examining how worm burden might change through a season or within years. Instead, proxy measures of parasite burden must be used. A common approach in both zoology and veterinary science is to use counts of worm eggs discharged in host faeces (faecal egg counts or FECs). There are a variety of established techniques for FECs that make use of the low density of the lipid-rich eggs. Samples are mixed with a flotation solution, commonly of salts in water, in which most organic faecal detritus sinks but nematode eggs float (Bowman & Georgi, 2009). The eggs can then be easily counted under a microscope. However, despite being widely used to assess worm burdens, FECs are not always reliable indicators of infection, and validating this reliability normally requires destructive sampling to match the proxy measure to *in situ* burden (e.g. Seivwright et al., 2004). The primary reason for this unreliability is that eggs in faeces result not from the presence of the parasite but its reproduction. Only adult worms produce eggs, so FECs do not account for larval worms. Larvae may make up a significant proportion of the overall burden, particularly in newly established infections (see below) such as we expect in chicks, and may cause more severe pathology than adults (Fagerholm & Overstreet, 2008). In addition, nematode species with different definintive hosts may nonetheless attach and cause damage but not reach a reproductive stage. Thus, not accounting for the

larval worm population may substantially underestimate the impact that an infection is having on the host. Furthermore, egg shedding may not occur at a steady rate (Shaw & Moss, 1989) or worm fecundity may be density-dependent (Tompkins & Hudson, 1999).

To overcome the caveats and difficulties of any proxy measure, we require direct measures of the worms themselves, rather than indicators of their presence. Necropsy is not possible in our shags, which are both protected and a long-term study population. Dissections are only possible using opportunistic recoveries of victims of natural mortality. In general, this sample is likely to be biased towards individuals of poorer quality or in poorer condition, and perhaps also towards individuals with high worm burdens if this contributed to their death (Hoberg, 2005; Fagerholm & Overstreet, 2008). This constraint has recently been overcome by the use of endoscopy to obtain direct counts of the worm burden in live adult shags (Burthe et al., 2013). This pioneering work demonstrated firstly that it is possible to view and count live parasites in a conscious bird, secondly that the technique was reliable and repeatable, and thirdly that treatment with ivermectin causes a lasting reduction in worm burden. This effect of treatment was only visible at doses higher than those used in this thesis, but anti-parasite treatment at lower levels has physiological effects both for chicks and parents (Reed et al., 2008, 2012; this thesis) and in chicks impairs worm reproduction as evidenced by a reduction in egg count (chapter 2). Endoscopy has not yet been carried out on chicks, either to assess the effects of treatment or to survey infection intensities in the population. These factors could contribute to our observed pattern that hatching order affects chicks' responses to anti-parasite treatment (Reed et al., 2012, chapter 2). Last-hatched chicks may have a different parasite burden to their older siblings, or be impacted to a different extent by a similar burden. In addition, we have shown that the impact of chick treatment on adults varies through the season (chapter 3). This could be a consequence of variation through the season in chick worm burdens, as there is in adult burdens (Burthe et al., 2013), or of variation in other extrinsic influences on the adults.

Here, we compare endoscopy, necropsy and FECs as measures of parasite burden in chicks. All work described in this appendix was carried out in 2012. We use necropsies from opportunistically recovered carcasses and endoscopy of live chicks to directly assess worm burden and, for endoscoped birds, the efficacy of anti-parasite treatment. We examine the role of rank, sex and hatch date on both the total burden of worms and the make-up of the worm community (relative prevalence of larvae and adults). Both

in situ measures are compared to egg counts of faecal samples taken at the same time to assess how well this proxy measure reflects a chick's real worm burden.

A.3.2 Methods

A.3.2.1 Endoscopy

We used a refurbished medical endoscope with a 9mm diameter (Olympus©UK; details in Burthe et al., 2013) to view the proventriculus and stomach of shag chicks. A trained specialist operated the endoscope while an assistant held the bird still and its bill open. A cloth was placed over the bird's eyes to reduce stress. The full endoscopy method is detailed in Burthe et al. (2013). The endoscope operator counted all worms that were visible and how many of these were adults. The operator also scored the visibility (from 1 to 4, worst to best), noting why the view was obstructed for poor visibilities. Endoscope work on adults has shown that worm counts obtained in this way are repeatable (Burthe et al., 2013).

The endoscopy work was conducted together with a parasite removal experiment, following the protocol in chapter 2. We visited nests of three eggs every two days around predicted hatching to obtain hatching dates. When the oldest chick in a brood was 10–12 days old, all three chicks in the brood were injected with either 0.05ml ivermectin (Panomec©by Merial, 1% wt/vol) or saline as a control. At this point ranks were assigned by mass, with the heaviest chick designated A, the middle B and the lightest C, and a blood sample taken for molecular sexing (Griffiths et al., 1996). This accurately identifies the last-hatched chick in over 90% of cases. Chicks were weighed at age 10 days, 15 days and 25 days to obtain growth rate by fitting a linear regression through the mass data for each chick.

We endoscoped all chicks from the experiment once large enough (66 chicks in 29 nests). In addition, we endoscoped chicks from unmanipulated nests whose hatch dates were also known to within two days. Together, endoscoped experimental and unmanipulated nests spanned the whole spread of phenology, including the first and last nests in this colony area to successfully fledge chicks in this year (hatch date range 25th April – 10th June, Julian dates 115–161). We first trialled endoscopy at day 10–12 and found it was not possible. Chicks were too small and their necks too bent to allow the endoscope comfortable passage. Endoscopy at this early stage was abandoned and trialled again at 5-day intervals to assess the viability of the technique for different sizes of chick. We found this to be age $\sim$25–30 days, with a minimum wing length of $\sim$100mm, and most endoscopy was carried out in this age range. We opportunistically

collected any faecal samples that chicks produced during endoscopy to obtain faecal egg counts (see below).

A.3.2.2 Dissections

In the middle of the peak chick-rearing period, there was a prolonged period of rain and cold weather. This resulted in considerable mortality due to waterlogging and chilling of chicks that were still downy (not yet waterproof) but too large to be efficiently sheltered by their parents. Mortality was thus not a direct consequence of overall poor condition nor of parasitism, though both factors may have contributed. Note that this sample (27 chicks) consists predominantly of chicks of an age that was most susceptible to the weather conditions (c.20–25 days old). Other opportunistic recoveries of dead chicks aside from this mortality event were also dissected (6 chicks). In total we obtained carcasses from 34 chicks, covering a range of hatch dates from 15th May to 11th June (Julian dates 135–162) and an age range of 12–45 days (75% in the range 17–27 days). We also dissected one adult carcass discovered opportunistically within a day of death. We used wing length to estimate chick age and to assign ranks in cases where the whole brood could be assessed (either dead or alive): the longest wing was assigned A and the shortest C. A sample of blood or tissue was taken from every carcass for molecular sexing (Griffiths et al., 1996).

Chicks were kept at $+4^{\circ}$C if they could be dissected within a day of being recovered. Otherwise, they were stored at -20°C and defrosted before being dissected up to a week later. The proventriculus and stomach were removed together with $\sim$3cm of oesophagus at the top and small intestine at the bottom. This was then opened out using one medial ventral cut and the contents examined in detail. The proventriculus is not obviously delineated from the oesophagus as there is no marked structural change. We defined this boundary as the point where the proventriculus reaches its narrowest point, which coincides with muscle folds becoming less pronounced. All worms were counted, removed and stored in ethanol, and later measured for length and separated by eye into age classes based on width. Large adults and small larvae were easily distinguished, and we also included an "unidentified" age class where there were doubts over a worm's classification. Once dissected, all carcasses were stored at -20°C. From these, a faecal sample was later taken from the cloaca to obtain faecal egg counts.

In the results section below, I present a quantitative analysis of patterns in worm burdens found in dissections followed by a descriptive account of observations from the dissections that may illuminate some aspects of the worms' biology.

A.3.2.3 Faecal egg counts (FECs)

We counted nematode eggs in faecal samples from both endoscoped and dissected chicks, all stored after collection at -20°C, using a flotation technique. The sample was defrosted and mixed well with 20ml concentrated salt solution per 1g of faeces. After most organic debris had settled (after c.10 minutes), three aliquots were taken from the top two-thirds of the water column and placed in McMaster slides (full details of protocol in chapter 2). Under each McMaster slide grid, 0.15ml (0.02g of faeces) was systematically searched for nematode eggs, identified morphologically, at 40x magnification. For each bird, the entire faecal sample was floated and 3 aliquots counted for eggs.

A.3.2.4 Statistical analysis

Worm burdens of endoscoped and dissected chicks were analysed both overall and accounting for the proportion of adult worms, which may indicate the maturity of the infection. For dissected chicks, we investigated worm volume as well as number, calculated from individual worm lengths and widths (age class). We excluded from all analyses one dissected chick that had been drug-treated (the only drug-treated dissection) and another that was anomalously old, late in the season and heavily infected, which was a male. We combined endoscoped and dissected chicks in an overall analysis comparing the two techniques. For this, we excluded drug-treated endoscoped chicks because they were not balanced in the dissected group.

Worm eggs in faecal samples were analysed both as presence/absence and as counts. In the count analysis, one chick with an extremely high count was excluded because of its very strong leverage (42 eggs, all other sample range 0–3 eggs). This chick was a female aged 35 days, hatched a week after the median and was the smaller of a brood of two. Faecal samples were obtained for endoscoped chicks in the age range 25–40 days, and for dissected chicks 12–45 days. Conversely, the range of hatch dates for faecal samples was narrower for dissected chicks (16th May to 11th June, Julian date, as shown on graphs, 136–162) than for endoscoped chicks (25th April to 10th June, Julian date 115–161). Because of this limited overlap in age and phenology of samples, our comparisons between endoscopy and dissection may not precisely compare like for like. Worms were present in all dissected chicks apart from the very youngest one (c.2 days old) and in all dissected chicks.

All analysis was done in R v2.15.1 (R Development Core Team, 2011), with nest fitted as a random variable in all models to account for non-independence of sibling. Counts were analysed using a poisson error distribution with a log link, presence/absence using a binomial error distribution and a logit link, and proportions using binomial errors weighted by the total worm count. All effect sizes are given as fitted with the link functions (not back-transformed). In endoscopy, worm counts were reduced by poor visibility (effect size 0.18±0.06 worms per score category, $z = 3.05$, $p = 0.002$), so visibility score was included in all models (proportions of samples in each category, from worst to best visibility: 1 – 13%; 2 – 19%; 3 – 51%; 4 – 17%). Models of the adult worm proportion in dissected chicks accounted for an effect of preservation method: the proportion of mature worms in chicks that had been frozen was 10% lower than in chicks that had been dissected fresh (effect size -0.71 ± 0.37, $z = -1.92$, $p = 0.055$). Also, a greater proportion of worms seen were adults if the chick had recently been fed, so this was included in all models of adult proportions (single main effect of recently fed: 1.54±0.59, $z = 2.63$, $p = 0.008$, but only if chick age also fitted). In dissected chicks, we could not formally test for both effect of age and hatch date in the same model. Most carcasses were collected after a single mass mortality event, and because wing length was used to calculate age and hence hatch date, age and hatch date were very closely correlated (in linear model, $r^2 = 0.72$, $p < 0.001$). Because hatch date was calculate from age and because we expect age to have a bigger influence on worm burdens than hatch date in the relatively narrow range this sample covers, we only examined the effects of age. We dissected one very young chick (c.2 days old) which had no worms at all; this was the only dissection that had a zero burden. This chick is excluded from all analyses because of its high leverage of both age and burden. In no cases did excluding the outlier change the direction of significant patterns.

A.3.3 Results & discussion

A.3.3.1 Endoscopy

Worm burden per chick varied from 0 to 30 worms (mean±std. err.: 10.7 ± 0.8). Older chicks had more worms (effect size 0.07±0.02, $z = 3.00$, $p = 0.003$; fig. A.1a) and, in addition to this, C chicks had a lower burden than their older siblings (in model including age and rank, compared to A chick, for B chicks: effect size −0.12±0.11, $z = -1.13$, $p = 0.260$; for C chicks: effect size −0.66±0.15, $z = -4.40$, $p < 0.001$;

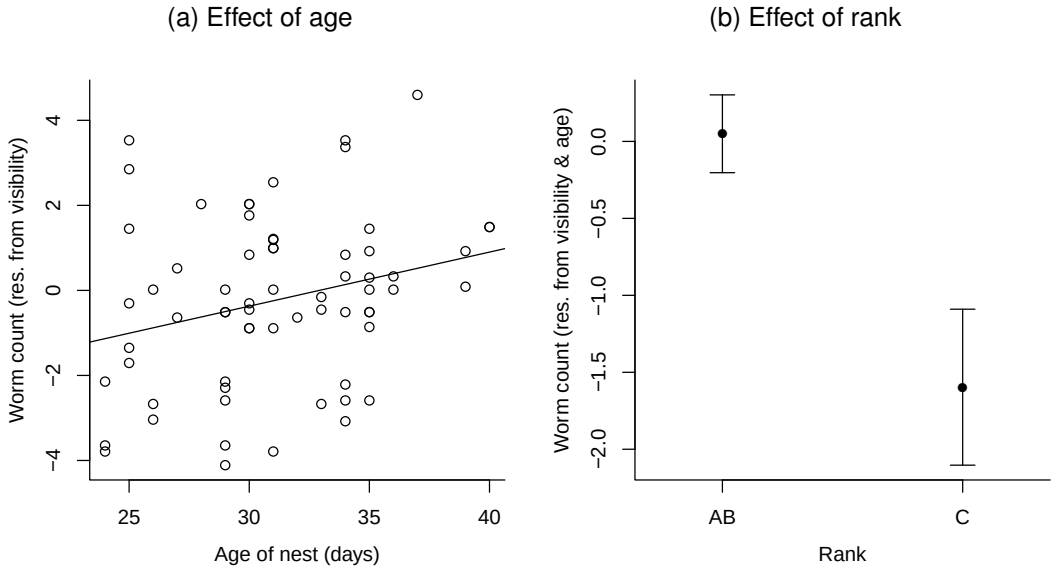


Figure A.1: Worm counts by endoscopy were higher for older chicks and lower for last-hatched siblings in a brood. A and B chicks did not differ so are pooled here for clarity, and ranks did not differ in their visibility scores ($\chi^2 = 1.14$, $p = 0.980$). Both graphs show the residual worm count from visibility scores.

fig. A.1b). Worm burden did not change through the season, sexes did not differ in their burden, and neither sex nor rank effects changed with age or through the season (main effects of hatch date and sex and all interactions $p > 0.8$).

Treatment with ivermectin tended to reduce chicks' worm burden, but not significantly (allowing for rank and age, treatment effect size -0.42 ± 0.23, $z = -1.88$, $p = 0.061$; fig. A.2). There was a weak suggestion that the treatment effect was driven by C chicks (compared to A chick, for B chicks: effect size 0.15 ± 0.22, $z = 0.71$, $p = 0.481$; for C chicks: effect size -0.89 ± 0.52, $z = -1.73$, $p = 0.084$). Treatment effect did not differ between the sexes, nor change with age or phenology (all $p > 0.1$).

In order to examine differences between chicks in the make-up of the parasite community, we also looked at variation in the proportion of worms that were adults. Older chicks had a higher proportion of adults (age effect 0.80 ± 0.13, $z = 6.40$, $p < 0.001$; fig. A.3a). This indicates that infections in chicks are established primarily by larvae which then mature, rather than by direct transmission of dislodged adult worms from parents. The proportion of adult worms increased slightly through the season (effect size 0.26 ± 0.10, $z = 2.47$, $p = 0.014$; fig. A.3b). In addition to age and phenology, rank affected adult proportion of worms in an unexpected way: B chicks had relatively

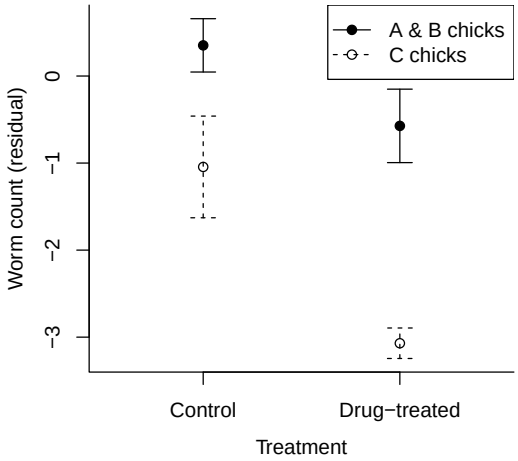


Figure A.2: Worm counts for endoscoped A & B chicks (solid symbols and lines) and C chicks (open symbols, dashed lines) in drug-treated and control broods. The effect of treatment and its interaction with rank were both marginally non-significant. The y-axis shows the residual worm count from fitting age and visbility. Sham-treated controls and unmanipulated nests are pooled in "controls".

fewer adults (compared to A chick, for B chicks: effect size -0.93 ± 0.41, $z = -2.29$, $p = 0.022$; for C chicks: effect size $+0.79\pm0.61$, $z = 1.30$, $p = 0.193$). Treatment did not affect the adult proportion of worms, in contrast to the total worm count (in addition to age, phenology and recent feed, treatment effect size -0.91 ± 1.52, $z = -0.60$, $p = 0.550$).

In the parasite manipulation experiment, chicks' growth rate was not affected by treatment, nor did this differ between ranks, sexes or through the season (treatment main effect and interactions all $p > 0.1$). This contrasted with 2010, when C chicks' growth rate was reduced by treatment, and 2011, when all chicks tended to grow slower in treated broods (chapters 2 and 3). These tests allowed for differences between ranks in growth rates: A chicks grew fastest and C chicks slowest (compared to A chick: for B chick, effect size -2.7 ± 1.5, $t = -1.87$, $p = 0.073$; for C chick, -7.7 ± 1.8, $t = -4.27$, $p < 0.001$). Unusually, in this year, males did not grow significantly faster than females (effect size 1.1 ± 1.6, $p = 0.498$). Worm burden as recorded from endoscopy was not linked to growth rate (as main effect in addition to rank, worm count and proportion adults and interaction all $p > 0.2$).

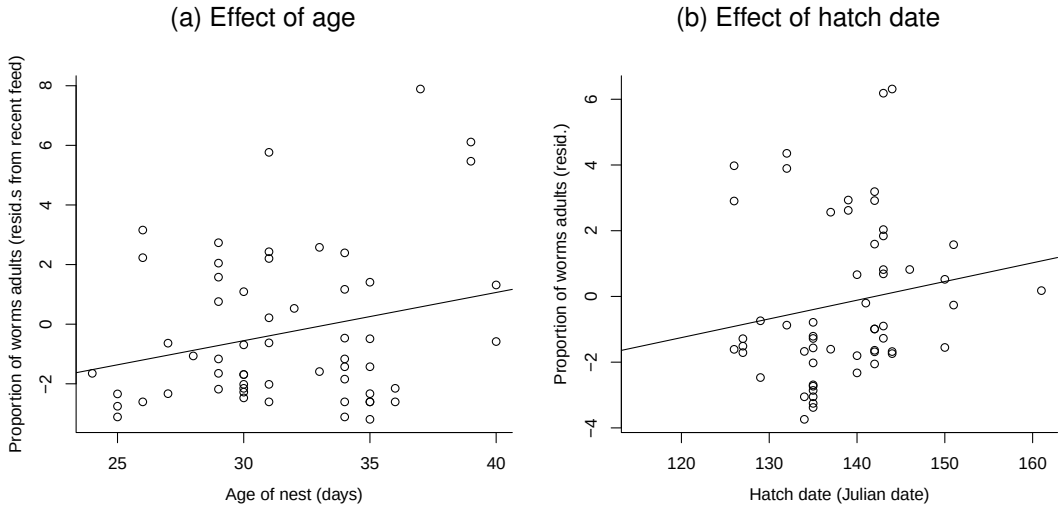


Figure A.3: The proportion of worms that were adults in relation to chick age (fig. A.3a) and hatch date (fig. A.3b) for endoscoped chicks. These graphs show residuals from whether a chick had been recently fed.

A.3.3.2 Dissections

Dissection yielded more variable worm burdens than endoscopy, with counts ranging from 8 to 243 in one chick (mean±std err., 41.5 ± 7.8), and covered a broader range of ages (12–45 days, compared to endoscopy 24–40 days). Apart from one 2-day-old chick, all dissected chicks hosted worms (youngest aged 12 days). Compared to endoscopy, dissection gave higher total worm counts and higher counts of adults (in addition to age, effect of method on total worm count: -2.08 ± 0.15, $z = -14.14$, $p < 0.001$, fig. A.4; on adult count: -1.79 ± 0.33, $z = -5.40$, $p < 0.001$). However, the measurement methods did not differ in the proportion of worms detected that were adults, indicating that both methods similarly represented the make-up of the nematode community (in addition to age, effect of method on proportion adults: 0.18 ± 0.42, $z = 0.43$, $p = 0.668$, fig. A.4). Differences between the measurement techniques in the following patterns of worm abundance are summarized in table A.1.

Older chicks hosted a larger number of worms, with worm burden increasing by 1.1 worms per day (effect size in poisson GLMM 0.06 ± 0.02, $z = 3.15$, $p = 0.002$; fig. A.5). In addition, there was an indication that male chicks hosted fewer worms than females throughout development, although this effect was not significant (main effect of sex: -0.18 ± 0.10, $z = -1.81$, $p = 0.070$; interaction with age, $p = 0.875$; fig. A.5). This contrasts with the pattern in adults, based on endoscopy, where males

Table A.1: Summary comparison of endoscopy and dissection for the effects of chick age, sex and rank on worm burdens, both in total and the proportion of worms that were adults.

Worm measure	Factor	Endoscopy pattern	Dissection pattern	Agreement?
Total count	Age	Older chicks more worms	Older chicks more worms	Yes
Total count	Sex	No effect	Males fewer worms (trend only)	Ambiguous
Total count	Rank	C chicks fewer worms	C chicks fewer in older chicks only	Broadly yes
Adult propn.	Age	Older chicks more adults	Not overall	Ambiguous
Adult propn.	Sex	No effect	Increases with age in males only	Ambiguous
Adult propn.	Rank	B chicks fewer adults	C chicks fewer in older chicks only	No
FEC presence	No. worms	No effect	Weak pos. association	Ambiguous

had higher burdens (Burthe et al., 2013). Chick rank did not affect total worm burden (main effect in addition to age and interaction with age, all $p > 0.3$).

The proportion of adult worms in dissected chicks increased with age in males but not in females (age * sex interaction: effect size 0.30 ± 0.09, $z = 3.40$, $p < 0.001$, fig A.6. In older siblings, adult proportion also increased with age, whereas it decreased in last-hatched chicks (rank * age interaction, compared to A chicks: for B chicks, effect size -0.04 ± 0.09, $p = 0.666$; for C chicks, effect size 0.23 ± 0.08, $z = 2.78$, $p = 0.005$; fig. A.7). Overall, adult proportion of worms did not change with age (effect size 0.02 ± 0.03, $p = 0.541$), in contrast to the pattern found using endoscopy. Only the role of rank, not sex, was mirrored in the proportion of worms unambiguously identified as larvae (age * rank on proportion larvae: for B chicks, $p = 0.933$, for C chicks, $p = 0.038$; age * sex interaction, $p > 0.1$).

The patterns in counts of worms and proportion of adults were reflected in patterns in total worm volume. Older birds had a greater volume of worms (effect size 9.0 ± 2.1, d.f.$= 12$, $t = 4.36$, $p = 0.001$) with a larger effect on adult worms than on larvae (effect of age on volume of larvae: 1.4 ± 0.5, $p = 0.014$; volume of adults: 7.1 ± 2.0, $p = 0.004$). There was a marginally non-significant trend for worm volume to increase

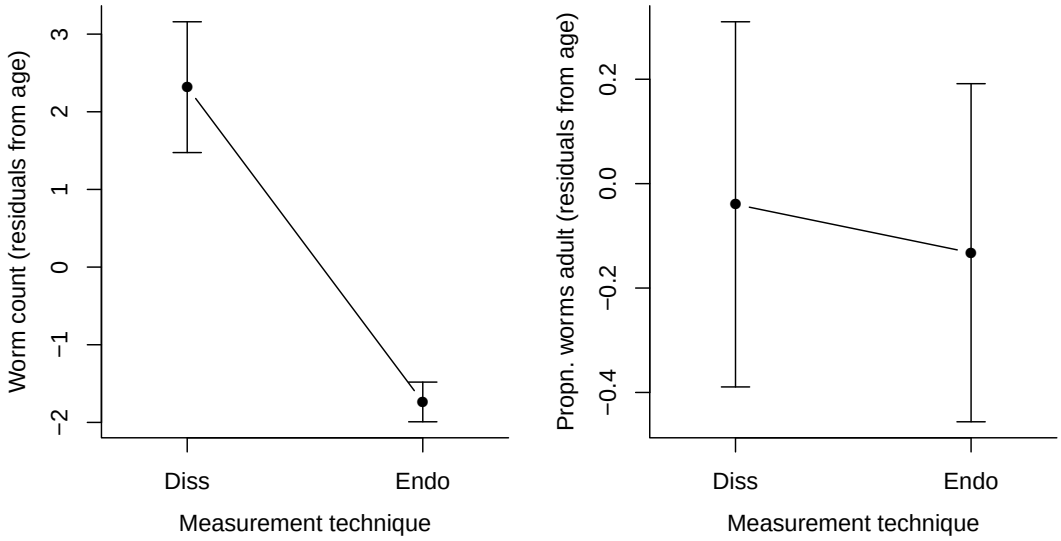


Figure A.4: A comparison of the two *in situ* measurement techniques, endoscopy and dissection, on overall worm count (left panel) and the proportion of worms that were adults (right panel).

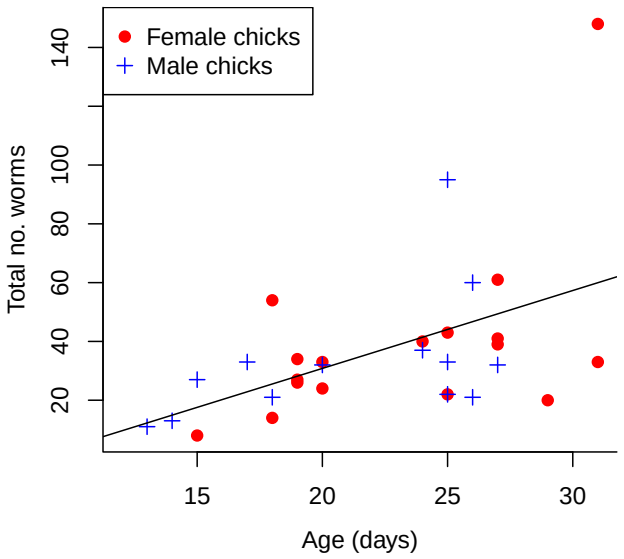


Figure A.5: In dissected chicks, the total number of worms in the proventriculus and stomach increased with age. This plot excludes the oldest and anomalously heavily parasitized bird dissected (45 days old, 243 worms) as this individual had very strong leverage on the pattern. Female chicks are shown in red solid circles and male chicks in blue open circles, but the model fit is presented for both sexes combined.

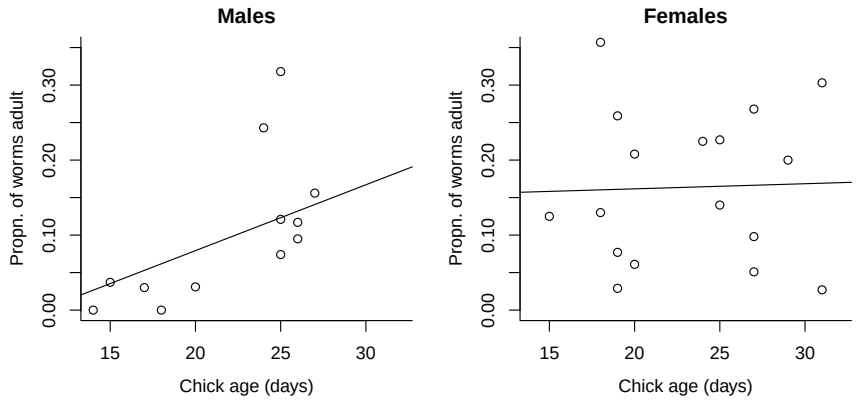


Figure A.6: In dissected male chicks, the proportion of worms that were adult increased with age, whereas in females, adult proportion of worms did not change with age.

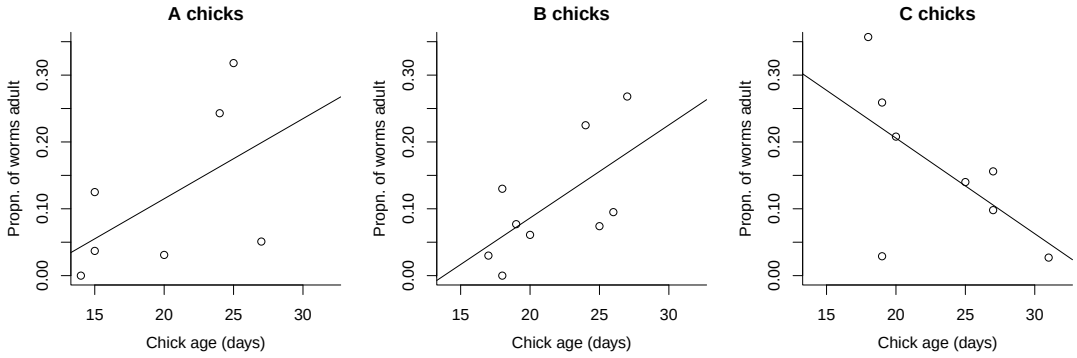


Figure A.7: In dissected chicks, the proportion of worms that were adult increased with age in older siblings but decreased with age in last-hatched chicks.

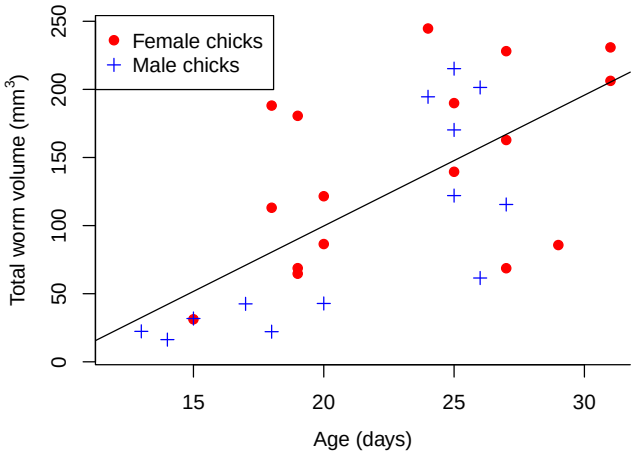


Figure A.8: Volume of worms increased with chick age, mirroring the effect on total number of worms. This plot excludes the oldest and anomalously heavily parasitized bird dissected (male, 45 days old, 1198mm^3 of worms) as this individual had very strong leverage on the pattern. Female chicks are shown in red solid circles and male chicks in blue open circles, but the model fit is presented for both sexes combined.

with age faster in male chicks than in females (age * sex interaction on total worm volume: effect size 7.1 ± 3.3, d.f.$= 10$, $t = 2.14$, $p = 0.058$; fig. A.8).

A.3.3.3 Dissection observations

During dissections, I observed signs of pathology, distribution of worms, their use of ingested food etc. to inform our understanding of their biology. All worms were dead by the time of dissection, apart from in 2 chicks where 2–3 worms were observed moving (dissected within 48 hours of death).

Only in two cases did I find worms above the proventriculus/oesophagus boundary. There were 3 and 6 individual worms in the oesophagus, and in neither case were there attachment points more than $\sim$ 1.5cm into the oesophagus. In no chick out of the 34 dissections did worms continue from the stomach into the intestine. In three chicks, I examined the whole intestine and found no visible helminths other than those in the proventriculus and stomach. In the adult intestine, we found 3 individual helminths (not nematodes) near the distal end. These are currently being identified morphologically. The burden of these was a fraction of that of the gastric nematodes (3 individuals, of a comparable size, compared to 56).

Within the stomach, bigger worms were found mostly at the top of the stomach and the smallest larvae predominantly at the bottom end. Middle-sized worms were

found all over the stomach. Moreover, food was more digested towards the bottom of the stomach. Thus, the smallest worms at the bottom of the stomach were often associated with a well-digested, homogenous paste of food. Adult worms were generally not found in this digested food, but on the outside of semi-digested boluses, and periodically also inside recently ingested food that was still identifiable as fish. This coincides with our observation in endoscoped chicks that relatively fewer adult worms were seen if the chick had been recently fed. This could indicate that mature worms are able to feed on less well digested food than the larvae, are therefore closely associated with recent food boluses and hence difficult to see with the endoscope. However, the most recently eaten fish (that were still mostly intact) had no worms associated with it, either inside or out. In most dissections, worms were also found in or even under the mucous lining of the stomach.

Where worms were, or had been, attached to the stomach wall, hardened ulcerations were sometimes evident. Larger worms were more frequently found attached than smaller worms, and often several worms (up to 13) were anchored in the same attachment scar. Multiple worms at the same attachment was not associated with a visible increase in the size of the ulceration or other damage. No swelling or other sign of infection was ever observed at an attachment point. Attachment point scars were all in the upper part of the stomach, more concentrated towards the oesophagus. This accords with the observation of bigger worms being associated with the upper regions of the stomach. There were far fewer attachment points than worms, indicating that the pathology does not arise in all cases of attachment and/or that the damage is repaired quickly. We do not have data on numbers of attachment points to formally test these hypotheses. Worms may benefit from attaching near the top of the stomach in order to be near newly ingested food, or alternatively, there may be less need further down to anchor themselves to prevent being dislodged during regurgitation. The decrease in attachment points towards the bottom of the stomach may also be associated with increasing thickness of the mucous lining, which could prevent effective attachment to the tissue underneath.

In chicks, worms are mainly larval, rarely over 25% adults. In sharp contrast, the dissected adult contained almost only adult worms (10% larvae). This difference between chicks and adults in the make-up of the worm community suggests that infections are established in chicks primarily by larval worms from fish, and hence that direct transmission via parents' regurgitated food is not a strong influence on a chick's parasite community. However, direct transmission probably does occur as eggs were

found in the faeces of chicks younger than the estimated maturation time of a newly-established L3 worm. Moreover, in a regurgitated food load from an adult which I examined for parasites, I found 4 larval and 5 adult worms. These have not yet been identified, but could be species unable to attach (e.g. *A. simplex* or *P. decipiens*) as well as *C. rudolphii* that is able to infect the chicks. This was a spontaneous regurgitation in response to stress, the contents of which may differ from what is given to a feeding chick.

The difference between the adult and chicks in worm count and the proportion of adults combined to a substantial difference in estimated worm volume that the birds were carrying. We do not have mass data for the dissected chicks, but we used wing lengths of dead chicks to estimate their mass from growth rates in this year. We assume that both worms and shags have a density of $1 g/cm^3$. Apart from the anomalously highly parasitized chick, worms made up a maximum of 0.03% of any chick's mass ($244 mm^3$ of worm in a 800g bird), and on average only 0.01%. The adult contained $951 mm^3$ of worms in a 2200g bird, i.e. 0.04% of its body mass, which was at the upper limit for the chicks.

A.3.3.4 FECs

Neither faecal egg presence nor counts varied with chick sex, rank or age in either endoscoped or dissected chicks or overall (as main effects or in interaction with age or hatch date, all $p > 0.1$). The youngest chick that passed eggs was 18 days old (in contrast to 2010, when eggs were present in the faeces of chicks only 8 days old; chapter 2). There was a non-significant trend for the likelihood of eggs being present in chick faeces (but not egg counts, for endoscoped chicks $p = 0.179$; for dissected chicks, $p = 0.511$) to change through the season, increasing with time for endoscoped chicks (effect size 0.11 ± 0.06, $z = 1.88$, $p = 0.061$; excluding drug-treated chicks, $p = 0.055$), but decreasing for dissected chicks (-0.22 ± 0.12, $z = -1.75$, $p = 0.080$) (fig. A.9). This could be due to the limited overlap in hatch dates between endoscoped and dissected chicks, such that the relationship between FECs and the two *in situ* measures may reflect different parts of a non-linear seasonal pattern.

From chicks included in the anti-nematode treatment study, we only obtained faecal samples for four endoscoped chicks treated with ivermectin, so testing the effect of treatment on the relationship between FECs and endoscopy worm count was not possible. Qualitatively, however, treatment appeared to reduce the likelihood of finding eggs: among control and unmanipulated chicks, eggs were absent in 10 and present

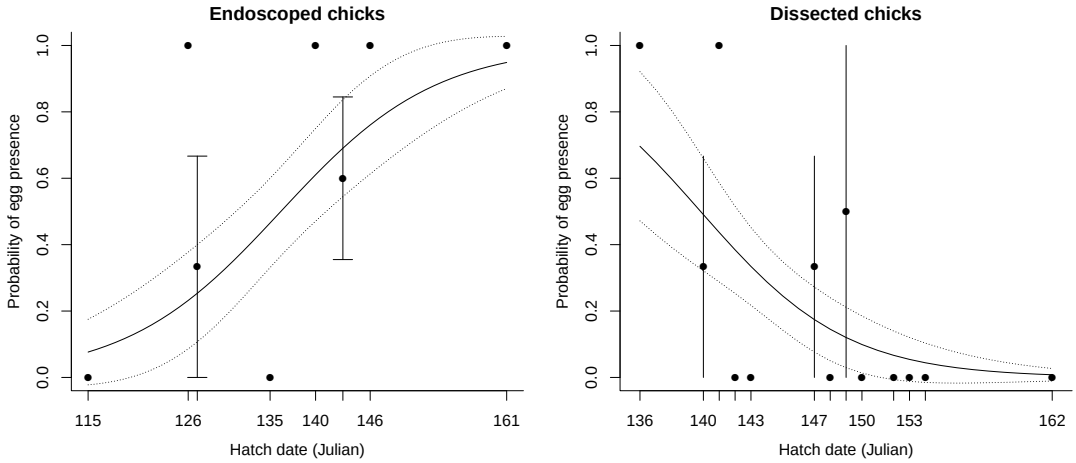


Figure A.9: Chicks hatched later in the season had higher faecal egg counts in samples from endoscoped chicks, but lower in samples from dissected chicks. Note that the earliest-hatched endoscoped chicks from which we obtained faecal samples hatched 20 days before the earliest-hatched dissected chicks with faecal samples.

in 9 ($47\pm12\%$ prevalence), whereas none of the drug-treated chicks had eggs present ($0\pm0\%$ prevalence). This accords with our findings from the 2010 season that treatment prevented an increase with age in egg presence (chapter 2).

In endoscoped chicks, neither faecal egg presence nor counts reflected the total worm burden or the adult worm burden (all $p > 0.1$). However, in the dissected chicks, there was a weak positive association between faecal egg presence and total worm burden (fig. A.10). Although the effect was not significant ($p = 0.12$), total worm burden explained variation in faecal egg presence better than an intercept-only model ($\Delta\text{AIC} = -2.0$). There was no effect on faecal egg presence of only adult worms, nor on faecal egg count of either total or adult worm burden (all $p > 0.3$).

Overall, egg presence in faeces was greater in live (endoscoped) chicks than in dead (dissected) chicks but only if total worm burden (not significant in itself) was taken into account (table A.2). There was no such effect on egg counts (all $p > 0.1$, all $\Delta\text{AIC} > 0.7$ from intercept-only model). When both dissected and endoscoped chicks were included, faecal egg presence increased with chick age, even though neither group separately showed this relationship (main effect size 0.13 ± 0.06, $z = 2.05$, $p = 0.040$). This was due to a link between total worm burden and age in both dissected and endscoped chicks. Allowing for a difference between the methods in the number of worms detected, faecal egg presence was as well explained by "total worm count + counting method" as by a main effect of chick age (table A.2).

Table A.2: A selection of models testing the influence of chick age, total worm burden and worm counting method on faecal egg counts across chicks sampled while alive (endoscoped) and after death (dissected). To illustrate the relative explanatory power of each model we present AIC weights. These were calculated from a fully balanced set of candidate models including all combinations of all three explanatory variables, but we present only the most informative models. Chick age was a significant main effect. In both endoscoped and dissected chicks, worm burden was correlated with chick age, yet total worm burden was not a significant factor in faecal egg presence across both groups. However, endoscopy detected fewer worms than dissection, so when counting method was included, total worm burden had a positive, though marginally non-signigicant, effect on faecal egg presence. Chick age did not show the same pattern. Because of the association between age and worm burden, a model including both was not informative. Chick age as a main effect had a very similar explanatory power to worm count + method.

Model	**Parameter**	**Estimate**	**z-value**	**p-value**	**ΔAIC from intercept-only**	**AIC weights**
Chick age		0.13±0.06	2.05	0.040	−2.7	0.275
Worm count		0.01±0.01	0.99	0.321	1.1	0.041
Method		1.00±0.71	1.41	0.159	0.1	0.069
Worm count + method					−2.4	0.241
	Worm count	0.04±0.02	1.73	0.083		
	Method	2.36±1.09	2.17	0.030		
Chick age + method					−0.7	0.101
	Chick age	0.13±0.08	1.63	0.103		
	Method	0.04±0.85	0.04	0.966		
Chick age + method + worm count					−1.3	0.072
	Chick age	0.09±0.09	0.97	0.332		
	Method	1.40±1.27	1.10	0.270		
	Worm count	0.03±0.02	1.38	0.168		

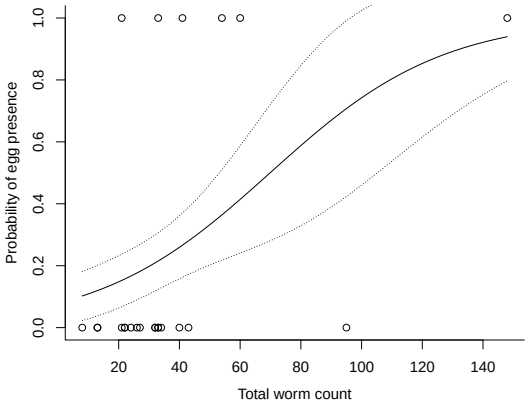


Figure A.10: In dissected chicks, presence of nematode eggs in faeces was weakly associated with the chicks' total worm burden. The relationship was not significant but did improve the explanatory power of the model.

Our results show that faecal egg counts in shags should be interpreted with caution but may be useful indicators of broad patterns in infection. We are not able to account for temporal variation in egg production as we are not aware of any work describing such patterns in *C. rudolphii*. Moreover, we expect FECs only to reflect *C. rudolphii* burdens, not *A. simplex*, which may potentially also impose costs on the shags but is unlikely to reach sexual maturity and thus produce eggs in birds. In addition, we have demonstrated a substantial risk for false negative FEC results: endoscopy and dissection showed that all chicks hosted worms, whereas 65% of faecal samples contained no eggs. This may be partly due to the low egg concentrations in this system; diagnostic veterinary protocols for ungulates require at least 2g of faeces and often many times more (Bowman & Georgi, 2009), whereas shag faecal samples are rarely over 1g in weight (average 0.5g in 2012). Lastly, it is worth noting that faecal samples from dissected chicks were taken from the cloaca after death. They may, therefore, be of a different composition to live exretions, potentially including different egg content.

A.4 Summary

In this appendix, we have synthesized and substantially expanded on current knowledge of the parasitology in the shag/nematode system. Discussion points are mainly covered in the text, and we summarize here our three main conclusions.

Firstly, we have pioneered the use of endoscopy for shag chicks and demonstrated the efficacy of chick treatment at dose levels used in this thesis. Patterns of infection in

relation to age, seasonality, sex and rank generally mirrored those found in dissected chicks, even though endoscopy underestimated total burdens, suggesting it is a reliable reflection of real parasite burdens.

Secondly, the route of infection of chicks seems to be predominantly with larvae from fish, rather than direct transfer of adults dislodged from parents. Some direct transfer probably occurs as chicks that were younger than the worms' maturation time hosted adult worms and produced eggs. However, chicks' nematode communities were dominated by larvae, in sharp contrast to the adult we dissected, and the proportion of adult worms in chicks increased with time, suggesting maturation of established larvae.

Lastly, worm burdens differ between chicks of different sex, rank and phenology, supporting various findings from this system of individuals differing in their response to anti-parasite treatment. In particular, rank effects were driven by C chicks, as are rank effects on the physiological impacts of anti-parasite treatment (chapter 2). However, C chicks generally had lower burdens, whereas based on their stronger responses to anti-parasite treatment we might expect the opposite. This suggests a potential role for within-clutch variation in maternal allocation of immunity, given that C chicks appear better able to resist infection but also pay higher costs of it. This illustrates the complex role that parasite infection plays in the ecology of the shag.

APPENDIX B

Details and extension of analysis for chapter 3: family-wide fitness impacts of parasitism

This appendix includes details of analysis from chapter 3. In that chapter, we investigate the impact of anti-parasite treatment of parents and/or chicks for all family members. We measure the effects of treatment for parents by examining their mass change through the chick-rearing period, and on chicks by examining their survival and growth rate.

In this appendix, we give details on two aspects of the analysis. Firstly, we address the derivation and application of background models of natural variation in each response variable examined, on top of which we tested for treatment effects. Secondly, we describe an investigation into the potential role of parent age in the observed phenological effects, as younger birds often breed later.

B.1 Background models

We derived background models for parent mass change, chick growth and chick survival to account for natural patterns in each response variable. Treatment effects were

tested in addition to these natural patterns, and observed treatment effects were robust to which background model was used (see section B.1.4 below). We derived background models for two sets of nests: the full data (including all experimental nests), in order to be sure of detecting subtle phenology patterns, and control-only nests, so as not to mistakenly attribute treatment effects to natural variation. Treatment effects were robust to which background model was used.

Background models for each response were selected from an initial full model of biologically reasonable predictions. This model was then simplified by sequential removal of non-significant terms until we reached a final, minimal model with all terms significant (table 3.1; model selection details in appendix B). All full background models included hatch date as the Isle of May shags display pronounced seasonal declines in success (e.g. Daunt et al., 1999; Reed et al., 2008). This could exacerbate the effects of other explanatory factors such as parent sex and chick rank, so hatch date was included in interaction with all other factors in the initial background model.

Background models were selected for each response variable by stepwise simplification from an initial full model of biologically reasonable predictions including, as applicable: hatch date, parent sex, chick sex and chick rank, and interactions of all factors with hatch date, as detailed in chapter 3. This full model was simplified by sequential removal of the least significant terms until we reached a final, minimal model with all terms significant. The model selection process is shown in table B.1 and minimal models for both groups of nests in table B.2.

B.1.1 Parent mass change

For parent mass change between the start and end of the treatment period, the full background model included parent sex, as mothers and fathers may have different trade-offs between investment in themselves and their offspring (Reed et al., 2008), in addition to hatch date. In this case, the full starting model was also the minimal model:

$$\text{Parent mass change} \sim \text{Sex} * \text{Hatch date} \; (+ \text{ Nest as random})$$

B.1.2 Chick growth rate

For chick growth rate, we included sex and rank in the full background model as males grow faster than females (Daunt et al., 2001b) and C chicks at a different rate to their older siblings (chapter 2). The initial and simplified background models were:

$$\text{Chick growth rate} \sim \text{Sex} * \text{Hatch date} + \text{Rank} * \text{Hatch date} \; (+ \text{ Nest})$$

$$\text{Growth rate} \sim \text{Sex} + \text{Rank} \; (+ \; \text{Nest})$$

B.1.3 Chick survival

For survival from parent treatment, the starting model was also the minimal model:

$$\text{Survival from parent treatment} \sim \text{Hatch date} \; (+ \; \text{Nest})$$

For post-hatching survival, the initial full model and simplified final model were:

$$\text{Post-hatching survival} \sim \text{Sex} * \text{Hatch date} + \text{Rank} * \text{Hatch date} + (+ \; \text{Nest})$$
$$\text{Post-hatching survival} \sim \text{Rank} * \text{Hatch date} \; (+ \; \text{Nest})$$

Most chick mortality occurs before chick age 10 days (Daunt et al., 1999) when chicks were treated in our protocol; in this experiment 23 chicks (14% of those hatched) died before treatment and only 11 (7%) after treatment. With so few deaths, our analysis of post-treatment mortality may have limited power. The initial and simplified background models for post-chick-treatment survival were:

$$\text{Post-treatment survival} \sim \text{Sex} * \text{Hatch date} + \text{Rank} * \text{Hatch date} + (+ \; \text{Nest})$$
$$\text{Post-treatment survival} \sim \text{Rank} + \text{Hatch date} \; (+ \; \text{Nest})$$

B.1.4 Application of background models

In chapter 3 we present treatment effects tested on top of the background models derived for all experimental nests. These captured subtle effects of hatch date that the "control-only" subset was too small to detect. However, treatment effects were qualitatively robust to which background model was used. This demonstrates that the "all-nests" background models were not attributing treatment effects to natural patterns of variation. Here, we demonstrate this robustness by showing key treatment effects tested on top of "control-only" background models. Table B.3 shows the analysis of the impact of chick treatment on parent mass change. Table B.4 shows the impact of parent treatment on chick survival. Table B.5 shows the effect of chick treatment on chick growth rate.

Table B.1: The derivation of background models on top of which treatment effects were tested. The starting model was simplified step-wise by removal of the least significant term. The p-value of that term is shown along with the ΔAIC from the preceding model once it was removed. The chapter analysis used models derived for all experimental nests as these captured the role of hatch date. In "control-only" nests, no chicks died after treatment, and only one control-treated chick died after treatment, hence a control-only background model for post-treatment survival could not be derived.

Starting model	**Term removed**	**p−value**	**Model ΔAIC**
Controls only			
Parent mass change ∼ Hatch date ∗ Sex			0.0
	Hatch date : sex	0.942	−2.0
	Hatch date	0.906	−2.0
	Sex	0.137	0.5
Survival egg−fledge ∼ Hatch date			0.0
	no term removed		
Post−hatching survival ∼ Rank ∗ Hatch date + Sex ∗ Hatch date			0.0
	Hatch date : sex	0.882	−2.1
	Hatch date : rank	0.475	−1.1
	Sex	0.827	−2.0
	Hatch date	0.129	1.0
Growth rate ∼ Sex ∗ Hatch date + Rank ∗ Hatch date			0.0
	Hatch date : rank	0.512	−3.4
	Hatch date : sex	0.735	−1.9
	Hatch date	0.464	−1.4
	Rank	0.205	−0.3
All experimental nests			
Parent mass change ∼ Hatch date ∗ Sex			
	no term removed		
Survival egg−fledge ∼ Hatch date			
	no term removed		
Post−hatching survival ∼ Rank ∗ Hatch date + Sex ∗ Hatch date			
	Hatch date : sex	0.106	1.4
	Sex	0.335	−0.9
Post-chick-treatment survival Rank * Hatch date + Sex * Hatch date			

Table B.1: The derivation of background models on top of which treatment effects were tested. The starting model was simplified step-wise by removal of the least significant term. The p-value of that term is shown along with the ΔAIC from the preceding model once it was removed. The chapter analysis used models derived for all experimental nests as these captured the role of hatch date. In "control-only" nests, no chicks died after treatment, and only one control-treated chick died after treatment, hence a control-only background model for post-treatment survival could not be derived.

Starting model	**Term removed**	**p−value**	**Model ΔAIC**
	Hatch date : sex	0.614	-1.8
	Sex	0.376	-1.2
	Hatch date : rank	0.252	-1.3
Growth rate $\sim$ Sex $*$ Hatch date + Rank $*$ Hatch date			
	Hatch date : sex	0.356	-1.1
	Hatch date : rank	0.127	0.4
	Hatch date	0.182	-0.1

Table B.2: Final, minimal background models describing natural variation in all response variables tested, fitted both to control nests only (to avoid misattributing treatment effects to natural variation) and to all nests (for a larger sample size to pick up potential interactions with hatch date). All models are (generalized) linear mixed models with nest fitted as the random factor. For survival models, the effect size is not back-transformed from the logit link function used for these binary response variables.

Response	**Background variables**	**Parameter estimate**	**p-value**
All nests			
Mass change	Sex $*$ Hatch date	-7.72 ± 3.37	0.026
Survival from egg	Hatch date	-0.08 ± 0.03	0.003
Post-hatching survival	Rank $*$ Hatch date	-0.35 ± 0.17	0.024
Post-treatment survival	Hatch date + Rank		
	Hatch date	-0.17 ± 0.05	0.001
	Rank (B chicks)	-1.08 ± 1.27	0.395
	Rank (C chicks)	-3.44 ± 1.23	0.005
Growth rate	Sex + Rank		
	Sex	$+3.27 \pm 0.55$	<0.001
	Rank (B chicks)	-0.01 ± 0.52	0.987
	Rank (C chicks)	-1.92 ± 0.66	0.004
Control nests only			
Mass change	(intercept only)	2.52 ± 21.0	0.906
Survival from egg	Hatch date	-1.68 ± 0.08	0.029
Post-hatching survival	Rank (B chicks)	-0.51 ± 0.99	0.605
	Rank (C chicks)	-2.41 ± 1.12	0.031
Growth rate	Sex	3.62 ± 1.00	0.002

Table B.3: The effect of chick and adult treatment on parents' mass change across the experimental period, tested on top of the background model derived for only control nests. AICs are presented compared to the model with the strongest treatment effect, i.e. Chick treatment * Hatch date. Note for the ΔAIC values that sex and hatch date are good non-experimental predictors of mass change (c.f. "all-nests" background model), hence models including a treatment interaction with these variables show substantial improvements to AIC without a significant treatment effect. For comparison, the fit of the strongest treatment effect model (Chick treatment * Hatch date) is improved by: adding Sex, $\Delta\mathrm{AIC} = -7.3$; adding Sex * Hatch date, $\Delta\mathrm{AIC} = -8.7$. We find the same significant treatment effects for this "control-only" background model as when using the "all-nests" background model: no impact of adult treatment, and an interaction between chick treatment and hatch date.

Parameter	**Estimate**	**t-value**	**p-value**	**Model ΔAIC from background**
Chick treatment	-26.0±23.8	-1.09	0.278	3.4
Treatment * hatch-date	-8.0±3.7	-2.14	0.037	0.0
Treatment * sex	-9.5 ±46.4	-0.20	0.839	-0.5
Treatment * sex * hatch date	-5.6±7.4	-0.76	0.449	-5.4
Adult treatment	-9.4±23.8	-0.40	0.694	4.4
Treatment * hatch date	-2.6±3.6	-0.72	0.474	4.6
Treatment * sex	67.2±46.3	1.45	0.154	-1.1
Treatment * sex * hatch date	-8.6±7.0	-1.24	0.223	-6.1
Adult treatment * chick treatment	-6.3±48.2	-0.13	0.897	7.3
Adult * chick treatment * date	-9.5±8.4	-1.13	0.263	4.5

Table B.4: Models testing the effect of adult treatment on chick survival, from egg to fledging and from hatching to fledging, on top of the "control-only" backgorund model. For survival from egg, this was a main effect of hatch date, and for post-hatching survival, a main effect of rank (table B.2). ΔAICs are presented relative to these background model. Chick sex and rank were only assigned at hatching so could only be tested on post-hatching survival. The AIC improvement for the last model is due to the explanatory power of the Rank * Hatch date interaction (c.f. "all-nests" backgorund model, table B.2), not an effect of treatment. For comparison, splitting this three-way interaction into its two important consitutent parts improves AIC further: for Treatment * Hatch date + Rank * Hatch date, $\Delta\text{AIC} = -15.4$.

Parameter	**Estimate**	**z-value**	**p-value**	**Model ΔAIC from background**
From egg				
Adult treatment (from egg)	0.07±0.40	0.18	0.857	2.0
Treatment * hatch date (cont.)	-0.11±0.06	-1.91	0.057	0.4
Post-hatching				
Adult treatment (post-hatching)	-0.13±0.64	-0.21	0.834	2.0
Treatment * hatch date (cont.)	-0.15±0.08	-1.79	0.074	-5.9
Treatment * hatch date (categ.)	-3.71±1.62	-2.28	0.022	-5.9
Treatment * sex	-0.40±1.08	-0.37	0.715	4.6
Treatment * rank	1.68±1.23	1.36	0.173	4.0
Treatment * sex *hatch date	0.27±0.16	1.69	0.091	-2.6
Treatment * rank * hatch date	0.07±0.49	0.14	0.886	-11.8

Table B.5: All models tested to investigate the effect of chick and adult treatment on chick growth rate, using the "control-only" background model: a main effect of sex. ΔAICs are presented relative to the best fit model (Chick treatment + Sex). Rank was an informative non-experimental variable for growth rate (c.f. "all-nests" backgorund model, table B.2), so some models including a treatment interaction with rank have improved AICs despite non-significant treatment terms. For comparison, adding rank to the model with the strongest treatment effect (Chick treatment main effect)achieves a ΔAIC of -5.2. The suggestion of a rank-dependent effect of adult treatment was driven by an absence of C chicks in later nests and the three-way interaction did not improve the explanatory power of the model (rank * hatch date model, $\Delta\mathrm{AIC} = -0.9$ compared to treatment * rank * hatch date model).

Parameter	**Estimate**	**t-value**	**p-value**	**Model ΔAIC from background**
Natural variation: Sex + Rank ∗ Date				
Chick treatment	-1.42±0.72	-1.98	0.053	0.0
Treatment * hatch-date	-0.08±0.11	-0.70	0.484	3.3
Treatment * sex	-0.92±1.12	-0.82	0.417	1.3
Treatment * rank (B chicks)	-0.54±1.05	-0.52	0.606	-1.9
Treatment * rank (C chicks)	0.46±1.31	0.35	0.725	
Treatment * sex * date	-0.17±0.19	-0.92	0.361	6.9
Treatment * rank * date (Bs)	-0.02±0.17	-0.13	0.895	2.1
Treatment * rank * date (Cs)	-0.33±0.30	-1.10	0.274	
Adult treatment	0.43±0.74	0.58	0.566	3.5
Treatment * date	-0.09±0.11	-0.84	0.403	6.0
Treatment * sex	0.15±1.14	0.13	0.893	5.5
Treatment * rank (Bs)	-0.82±1.05	-0.79	0.434	0.5
Treatment * rank (Cs)	0.42±1.32	0.32	0.752	
Treatment * sex * date	-0.04±0.18	-0.25	0.807	10.7
Treatment * rank * date (Bs)	0.38±0.14	2.63	0.011	-3.5
Treatment * rank * date (Cs)	-0.01±0.30	-0.04	0.968	
Adult treatment * chick treatment	0.93±1.44	0.65	0.521	3.2
Adult * chick treatment * date	-0.17±0.24	-0.69	0.495	6.8

B.2 Role of parental age

In this closely monitored population, the exact age was known for over half of all experimental parents (ringed as chicks), and a minimum age for the rest (ringed as adults, assumed to be first-time breeders and hence 3 years old at ringing). We tested whether hatch date effects were driven by differences in parental age as older birds tend to breed earlier and younger birds later (Daunt et al., 1999). Older, more experienced parents breed earlier and are more successful at chick-rearing (Daunt et al., 1999, 2001b) and might also be better able to preserve their own condition through chick-rearing.

First, we tested whether minimum age was an accurate proxy for true age. Adult ringing effort has varied between years, so some birds may have been breeding for several years before they were ringed, i.e. older than 3 years. However, restricting all analyses of age to exact ages would severaly restrict sample size. For each response variable, we tested whether exact age explained any of the variation accounted for by hatch date (table B.6). No effects were found for exact age that were not captured by hatch date, nor did age explain any response better than hatch date. This indicated that age was not well correlated with hatch date in our experimental nests. Therefore, we fitted background models to the best estimate of age available for each individual, i.e. a mixture of minimum and exact ages, so that all individuals could be included. None of the response variables varied with parent age (table B.7).

Table B.6: Models demonstrating the lack of effect of parent age using only birds of known age. In all models, hatch date and/or age as shown were included in the background model for each response variable, including interactions with factors such as sex and, for chicks, rank. AICs are shown relative to the background model using only hatch date. For nests where both parents were of known age, these models use the average (17 nests, correlation between mother's and father's age: $r^2 = 0.33$, $p = 0.010$). Where there was an effect of hatch date, parent age did not show any similar influence, and in no case did parent age fit the data better than hatch date.

Response	**Explanatory variables**	**p-values**	**Model ΔAIC**
Parent mass change	Hatch date	0.267	0
	Hatch date + age	0.575; 0.406	1.3
	Age	0.206	-0.3
Fledging success	Hatch date	0.001	0
	Hatch date + age	0.013; 0.597	1.7
	Age	0.035	5.9
Chick growth rate	Hatch date	0.196	0
	Hatch date + age	0.390; 0.586	1.7
	Age	0.270	0.5

Table B.7: Derivation including parental age of the background models for variation in all response variables measured. Models are presented sequentially in the order in which terms were removed. p-values are of the term in question, and ΔAICs show the change once that term was removed. Background models were derived for nests with only control adults and chicks and separately for all experimental nests. Phenology is fitted as continuous hatch date.

Term removed	p-value	Model ΔAIC
Control nests only		
Parent mass change ∼ Phenology * Sex + Phenology * Age		0.0
Phenology : age	0.428	-1.2
Phenology : sex	0.884	-2.0
Phenology	0.935	-2.0
Age	0.783	-1.9
Sex	0.137	0.5
Survival egg-fledge ∼ Parent age * Phenology		0.0
Phenology : parent age	0.431	-1.4
Parent age	0.251	-0.7
Post-hatch survival ∼ Rank * Phen. + Sex * Phen. + Parent age * Phen.		0.0
Phenology : sex	0.935	-2.1
Phenology : rank	0.571	-0.5
Sex	0.773	-1.9
Phenology	0.161	0.7
Growth rate ∼ Sex * Phen. + Rank * Phen. + Parent age * Phen.		0.0
Phenology : parent age	0.603	-1.6
Phenology : sex	0.540	-1.5
Parent age	0.702	-1.8
Phenology : rank	0.847	-3.6
Rank	0.243	-0.6
All experimental nests		
Parent mass change ∼ Phenology * Sex + Phenology * Age		0.0
Phenology : age	0.410	-1.3
Age	0.359	-1.1
Survival egg-fledge ∼ Parent age * Phenology		0.0
Phenology : parent age	0.294	-0.9

Table B.7: Derivation including parental age of the background models for variation in all response variables measured. Models are presented sequentially in the order in which terms were removed. p-values are of the term in question, and ΔAICs show the change once that term was removed. Background models were derived for nests with only control adults and chicks and separately for all experimental nests. Phenology is fitted as continuous hatch date.

Term removed	**p-value**	**Model ΔAIC**
Parent age	0.246	-0.7
Post-hatch survival ∼ Rank * Phen. + Sex * Phen. + Parent age * Phen.		0.0
Phenology : parent age	0.193	-0.1
Phenology : sex	0.165	0.7
Sex	0.223	-0.3
Age	0.199	-0.1
Growth rate ∼ Sex * Phen. + Rank * Phen. + Parent age * Phen.		0.0
Phenology : parent age	0.876	-2.0
Phenology : sex	0.357	-1.1
Parent age	0.210	-0.3
Phenology : rank	0.129	0.3
Phenology	0.184	-0.2

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